AD_____

Award Number: W81XWH-12-1-0441

TITLE: Pathogen-Reduced, Plasmalyte-Extended Stored Platelets

PRINCIPAL INVESTIGATOR: Sherrill J Slichter, MD, Director of Platelet Transfusion Research, Puget Sound Blood Center, Seattle. WA

CONTRACTING ORGANIZATION: Puget Sound Blood Center
Seattle, WA 98104

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2013	Annual	15 September 2012–14 September 2013

4. TITLE AND SUBTITLE
Pathogen-Reduced, Plasmalyte-Extended Stored Platelets (PREPS)

5a. CONTRACT NUMBER
W81XWH-12-1-0441

5b. GRANT NUMBER W81XWH-12-1-0441

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sherrill J. Slichter, MD

E-Mail: sherrills@psbc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Puget Sound Blood Center, 921 Terry Ave., Seattle, WA 98104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This grant pertains to finding novel approaches for storage of platelets for transfusion. Our project proposes to determine the efficacy of using a pathogen inactivation technique (Mirasol) coupled with a platelet storage solution (Plasmalyte) to extend the life of stored platelets. Our project also aims to determine how long acceptable platelet viability can be maintained in platelets derived from whole blood stored at 4°C. We have completed the regulatory activities necessary to initiate enrollment in the study of platelets in whole blood. We have developed the documents (IRB application, radiation safety application, consent etc.) for submission of the Mirasol/Plasmalyte portion of the study.

15. SUBJECT TERMS
apheresis, bleeding, extended storage, hemorrhage, hemostasis, Mirasol, pathogen inactivation, pathogen reduction technology, Plasmalyte, platelet recovery and survival, platelet storage, platelets, thrombocytopenia, transfusion, whole blood

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	65	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Pathogen-Reduced, Plasmalyte-Extended Stored Platelets (PREPS)
Grant Number 11105004
Annual Report
15 Sep 2012 – 14 Sep 2013

INTRODUCTION: The purpose of this project is to find better ways to store platelets for patients that need platelet transfusions. A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research study, we are interested in evaluating the novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on platelet viability and function following transfusion.

BODY: The following specific aims were described in the original statement of work, Novel Approaches to Storage of Platelets for Transfusion.

1. Determine the acceptable storage duration of radiolabeled autologous pathogen-reduced Plasmalyte stored apheresis platelets.
2. Determine the post-transfusion radiolabeled platelet recovery and survival of extended Plasmalyte stored pathogen-reduced donor apheresis platelets given to thrombocytopenic patients.
3. Evaluate the in vivo hemostatic efficacy of pathogen-reduced extended Plasmalyte stored platelets by determining the relationship between post-transfusion bleeding times and platelet counts in thrombocytopenic patients.
4. Assess the hemostatic efficacy, post-transfusion platelet responses, alloimmunization rates and adverse events following transfusion of extended Plasmalyte stored pathogen-reduced platelets.

As a first step we are initiating an evaluation of changes in the structural and functional properties of platelets stored as whole blood under refrigeration (Brrr Study). This preliminary study was determined by the Grant Officer Representative/Contracting Officer Representative (GOR/COR) at TATRC to be a crucial initial step to provide data to evaluate platelet storage duration, safety and efficacy improvements.

During this reporting period we obtained approval to conduct the Brrr study from our local IRB, our local radiation safety committee, TATRC and HRPO. We anticipate the first subject enrolments to occur in October.

Our prepared IRB application documents pertaining to Aim #1 above are awaiting an IDE prior to submission to TATRC and our local IRB.

KEY RESEARCH ACCOMPLISHMENTS: The following are the key research accomplishments to date.

<u>Brrr Study</u>

- Protocol and other regulatory document developed
- Laboratory equipment and reagents purchased
- Test methodology refined for cold stored platelet radiolabeling, aggregation and thromboelastograph/ROTEM
- Radiation Safety approval
- Telemedicine & Advanced Technology Research Center (TATRC) approval
- University of Washington's (UW) Human Subject's Division (IRB) approval
- USAMRMC Office of Research Protections (ORP HRPO) approval

<u>Aim #1 - Pathogen-Reduced, Plasmalyte-Extended Stored Platelets (PREPS)</u>

- Protocol and other regulatory document developed

REPORTABLE OUTCOMES: There are, as yet, no reportable outcomes.

CONCLUSION: We anticipate initiation of enrollment in both the Brrr study and the PREPS study in the next reporting period.

REFERENCES: None

APPENDICES:

- Protocol Brrr
- Consent Brrr
- IRB and HRPO approval notices
- Radiation Safety approval notice
- Clinical Report Forms

SUPPORTING DATA: None

Puget Sound Blood Center	
Title: Assessment of Whole Blood Cold Stored Platelets (Brrr Study)	
Puget Sound Blood Center Protocol: #12-10	**Version:** 1
Author: Sherrill J. Slichter, MD	**Rev Date:** 07/17/2013

Investigators

Sherrill J. Slichter, MD
Director of Platelet Transfusion Research Puget Sound Blood Center
921 Terry Avenue
Seattle, WA 98104

Professor of Medicine
University of Washington School of Medicine

Sponsor

Department of Defense (DOD)
Telemedicine & Advanced Technology Research Center (TATRC)
U.S. Army Medical Research and Materiel Command (USAMRMC)

PROTOCOL SIGNATURE PAGE

Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

Protocol: PSBC-12-10-DRAFT

Revision Date: 17th July 2013

I have read this protocol and agree to adhere to its requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the investigational device. I will ensure that the study is conducted in compliance with the protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements, and to the Institutional Review Board (IRB) requirements.

_____	_____
Investigational Site Name	Site Location (City, State)

_____

Principal Investigator Printed Name

_____	_____
Principal Investigator Signature	Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROTOCOL SYNOPSIS

Protocol Name: Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
Protocol Number: PSBC #12-10		
This study is planned to be conducted in accordance with the Declaration of Helsinki, the current International Conference on Harmonization – Good Clinical Practice (ICH-GCP) guidelines, and according to US CFR Title 21 applicable regulations.		
Investigator: Sherrill J. Slichter, M.D.		
Study Centers: Puget Sound Blood Center, Seattle, WA		
Study Period:	12 months	**Phase of Development:**
Planned Date of First Subject Enrolled:	October 1, 2013	Feasibility in Healthy Humans
Planned Date of Last Subject Enrolled:	October 1, 2014	
Date of Last Follow Up:	November 30, 2014	

Purpose of the Study:
To evaluate radiolabeled recovery and survival of autologous platelets in healthy adult subjects whose platelets have been derived from their whole blood donations that have been stored at 4°C for up to 21 days.

Objectives:
To determine how long acceptable platelet viability can be maintained in platelets derived from whole blood stored at 4°C.

Other Data Collection:
Samples of the whole blood will be obtained pre and post storage to evaluate platelet function using various in-vitro assays.

Methodology:
Subjects will be enrolled at the Puget Sound Blood Center and will donate one unit of whole blood. This unit will be stored at 4°C for a predefined storage period. A variety of *in vitro* metabolic and functional platelet assays will be performed on the stored unit.

***In Vivo* Autologous Radiolabeled Platelet Recoveries and Survivals**
After the storage period, an aliquot from the whole blood unit will be processed to obtain platelets. The subject will return and donate a fresh sample of whole blood (43 mls) from which a fresh aliquot of platelets will be obtained. The stored and fresh platelets will each be labeled with a different radioisotope (either Chromium-51 or Indium-111) and reinfused into the subject. Using two different radioisotopes enables separate tracking of both the stored and fresh platelets. Follow-up blood samples will be drawn at 2 hours and on days 1, 2, 3, 5, 7±1 and 10±2. Stored and Fresh platelet recoveries and survivals will be calculated.

Number of Subjects (Planned):
Healthy adult subjects will be enrolled until 8 subjects have complete recovery and survival evaluations for each selected storage time between 4 and 21 days (a total of 56 subjects). It is anticipated that up to 80 subjects may need to be enrolled to obtain complete evaluations to meet study endpoints.

Main Criteria for Inclusion:

Initial Inclusion:

Healthy adult subjects who meet standard blood donation requirements (travel exclusions and virology screening will not apply).

Inclusion for radiolabeled reinfusion of platelets:

Maintenance of healthy status (i.e., no flu or cold symptoms developed since donation, no other change to health).

Confirmation of negative pregnancy status in females.

Test Product:

Transfusion Blood Component: Platelets derived from CPD anticoagulated whole blood that has been stored for up to 21 days at 4°C.

Duration of Treatment:

Whole blood: Whole blood units, collected in CPD will be held for 4 to 20 days at 4°C. A variety of *in vitro* metabolic and functional platelet assays will be performed on the stored unit.

Subject Involvement: Subjects will be screened, enrolled and will donate one unit of whole blood. They will return at the end of storage (Day 0) (date assigned at time of consent) for reinfusion of their radiolabeled autologous platelets derived from the stored whole blood and for simultaneous reinfusion of their fresh platelets. They will have blood samples drawn prior to and after the reinfusion of their radiolabeled platelets. Subjects will return for blood sampling between 1 and 2 hours after infusion and on Days 1, 2, 3, 5, 7 ± 1, and 10 ± 2 post infusion. After these visits, the subjects' participation will end and they will exit the study.

Criteria for Evaluation-Primary Endpoint*:*

Prior studies have indicated that platelets stored as concentrates at 4°C for up to 3 days have relatively normal recoveries but very short survivals[1]. Therefore the primary endpoint of this study will be to determine the relationship between each donor's fresh and stored platelet recoveries. The lower 95% confidence limit for the data should be that platelet recoveries at the end of storage should be ≥50% of fresh and survivals should be ≥ 1 day.

Statistical Methods:

We will calculate means, standard deviations and confidence limits of stored and fresh recoveries and survivals. Within each subject we will calculate the difference between stored recovery and 0.50 x fresh recovery. Confidence limits for the mean of this difference will be calculated. All means will be plotted against whole blood storage interval.

1 PROTOCOL TITLE

Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

2 PHASE

This study is planned as a Phase I/II 'Proof of Principle' study to gather preliminary evidence about the viability of platelets derived from whole blood units that have been stored at 4°C for between 4 and 20 days. Based upon the results from this trial, clinical studies in thrombocytopenic patients to evaluate platelet viability and function, the latter as assessed by bleeding time measurements, will be performed.

3 PRINCIPAL INVESTIGATOR / STUDY STAFF

Sponsor:	Department of Defense (DOD) Telemedicine & Advanced Technology Research Center (TATRC) U.S. Army Medical Research and Materiel Command (USAMRMC)
Principal Investigators:	Sherrill J. Slichter, M.D. Director, Platelet Transfusion Research Puget Sound Blood Center 921 Terry Avenue Seattle, WA 98104-1256 Phone: (206) 292-6541 sherrills@psbc.org
Data Coordinating Center and Statistician:	Doug Bolgiano, MS Puget Sound Blood Center, Research Institute 1551 Eastlake Avenue East Seattle, WA 98102-3706 Phone: (206) 568-2235 dougb@psbc.org

4 STUDY LOCATIONS

Whole blood collections and platelet recovery and survival evaluations will occur at the Puget Sound Blood Center, under the direction of Dr. Sherrill J. Slichter. Bacterial cultures and Gram staining will be conducted by the University of Washington Microbiology Laboratory. Thrombelastograph (TEG) samples will be evaluated by the University of Washington Coagulation Laboratory. Additional samples for coagulation, complement microparticle analysis will be sent to the Blood Research Program, US Army Institute of Surgical Research (ISR), Fort Sam Houston, Texas.

5 ESTIMATED TIME REQUIRED TO COMPLETE THE STUDY

First subject enrollment planned: Q3 2013
Last subject enrollment planned: Q4 2014
Completed data analysis and report: Q2 2015

6 INTRODUCTION

6.1 BACKGROUND

Platelets are transfused to prevent bleeding and induce hemostasis, and can thus be critical in saving lives following trauma. Currently, platelets isolated from volunteer donors are stored at room temperature with gentle agitation for up to 5 days before transfusion. This short shelf-life severely compromises platelet inventories and creates chronic shortages for two important reasons: (1) platelets age during this period, and are functionally not as desirable as fresh platelets; and (2) storage at room temperature increases the risk of bacterial contamination. There is an urgent need to develop novel methods of storing platelets to minimize or even eliminate these issues. This need is particularly acute in the deployed military setting where platelet products are in especially short supply and are essentially unavailable far-forward, near the point of injury where they might be of greatest utility.

Fractionation of whole blood (WB) into components (red blood cells, plasma and platelets) was developed to more efficiently treat specific deficiencies and to facilitate storage of the blood components. US combat casualty care practice has increased the survival rates of massively injured soldiers and civilians, in part due to changes to resuscitation practices that include component transfusion strategy of 1:1:1 (RBCs:plasma:platelets) and the use of warm fresh whole blood (FWB). Current practice guidelines by the military are to transfuse stored tested blood components, if available, in a 1:1:1 ratio, similar to the composition of WB for patients with life-threatening bleeding. If any of the components are not available, warm FWB is to be used to supplement the unavailable component[2] or, if the 1:1:1 strategy is failing, the use of warm FWB is permitted. Such intensive plasma-based therapy early in resuscitation has led to a 50% reduction in mortality in massively transfused trauma patients.[3,4] However, if all of the components necessary for resuscitation could be stored as whole blood without fractionation into components, this would greatly simplify forward combat resuscitation strategies.

Storage of whole blood under refrigeration may maintain important aspects of platelet function. This was once standard-of-care in transfusion medicine, but was abandoned once it was shown that refrigeration led to accelerated *in vivo* platelet clearance over about 48 hours rather than over one week. While not conducive to maintaining circulating platelet counts in thrombocytopenic cancer patients, transfusion of fresh whole blood in the field might provide adequate platelet hemostatic capacity to bleeding trauma patients and improve platelet availability for such patients. This possibility has been inadequately evaluated, particularly in clinical studies.

A deeper understanding of the effects of cold storage on platelet function could greatly aid in improving the availability of platelets on the battlefield and in the civilian transfusion setting. In this research proposal we are interested in evaluating metabolic, functional and viability changes to platelets preserved in whole blood (WB) and stored at 4° C. We will also determine the recovery and survival of these platelets by radiolabeling an aliquot of the WB derived platelets and re-infusing it into the donor/subject.

6.2 MILITARY USE OF REFRIGERATED WHOLE BLOOD

This product is amenable for far-forward military trauma response situations. It will simplify handling for the clinician, save costs, and reduce donor exposures.

6.3 RATIONALE FOR PROPOSED STUDY

This proposal will determine how long platelets stored within whole blood at 4° C will maintain acceptable post transfusion platelet viability.

6.4 SUMMARY OF RELEVANT CLINICAL STUDIES

There have been no prior studies done to evaluate in-vivo platelet viability when platelets are stored within whole blood at 4° C.

Dr. Cap has data suggesting that in vitro measurements of platelets stored for up to 12 days at 4°C in whole blood are relatively stable. The Department of Defense is supporting radiolabeling studies of red cells that have been stored for 21 days in pathogen reduced whole blood (Mirasol Technology). Depending on the result of these studies, additional studies may be performed to evaluate viability of platelets stored in whole blood that has been previously pathogen reduced.

In most platelet storage studies, 8-10 measurements of the ‘test conditions’ are sufficient to provide information to achieve a reliable outcome.

7 OBJECTIVES / SPECIFIC AIMS / STUDY QUESTIONS

The purpose of our study is to evaluate viability and functional characteristics of platelets derived from whole blood that has been stored for up to 21 days at 4°C. The viability criteria of primary interest are whether WB stored platelets have recoveries of 50% of fresh and survivals of at least one day. Performance criteria will be evaluated in healthy subjects, who receive radiolabeled, autologous infusions. We will also evaluate function based on *in vitro* testing of the stored whole blood unit for platelet count, platelet functional assays, and platelet activation and apoptotic markers.

7.1 PRIMARY ENDPOINT

The purpose of this study is to determine the *in vivo* post-storage viability and *in vitro* function of platelets stored in whole blood at 4° C. It is anticipated that the primary use of 4° C stored whole blood will be to provide RBCs, plasma and platelets in forward combat situations without the need to separate the blood into components. Therefore these platelets need to provide a reasonable post-transfusion platelet increment, survival of at least one day, and evidence of function for at least 6 hours to maintain hemostasis until surgical repair can be performed.

The FDA has indicated that for room temperature stored platelets that are predominantly given to chronically thrombocytopenic patients, the radiolabeled autologous recovery and survival of their stored platelets should be compared to their fresh platelets because of the known heterogeneity between normal subjects' platelet viability. The criteria they have established are that the lower 95% confidence limit for stored recoveries should be 66% of the same subject's fresh recoveries and stored survival should be 58% of fresh. However, traumatized soldiers are likely to need only transient platelet support. Therefore, we are proposing that the 95% lower confidence limits for recoveries should be ≥50% of fresh and survivals should be at least one day.

We will evaluate 8 healthy subjects at WB storage times between 4 and 20 days. We will calculate means, standard deviations and confidence limits of fresh and stored recoveries and survivals and of the difference between stored recovery and 0.50 x fresh recovery.

8 HYPOTHESIS

We can store platelets for ≤21 days at 4°C in Whole Blood and meet our acceptance criteria.

9 STUDY DESIGN

Subjects will be enrolled for evaluation of platelet recovery and survival at the Puget Sound Blood Center. Healthy subjects will be consented and enrolled into the study until 8 subjects have completed the recovery and survival evaluations at 4, 8, 12, 16, and 20 days storage. The order in which storage intervals are evaluated will be randomized. Eight subjects will then be evaluated at the mid-range interval between longest storage period that meets acceptances criteria and shortest storage period that does not meet acceptance criteria. For instance, if at 12 days of storage the 95% lower confidence limits for recoveries are NOT ≥50% of fresh and survivals are LESS than one day, but at 8 days of storage recoveries are ≥50% of fresh and survivals are at least than one day, we will then evaluate 10 days of storage. If the 10 day storage does not meet acceptance criteria we will evaluate 9 days. If the 10 day storage does meet acceptance criteria we will evaluate 11 days. Eight subjects will be evaluated at each storage interval.

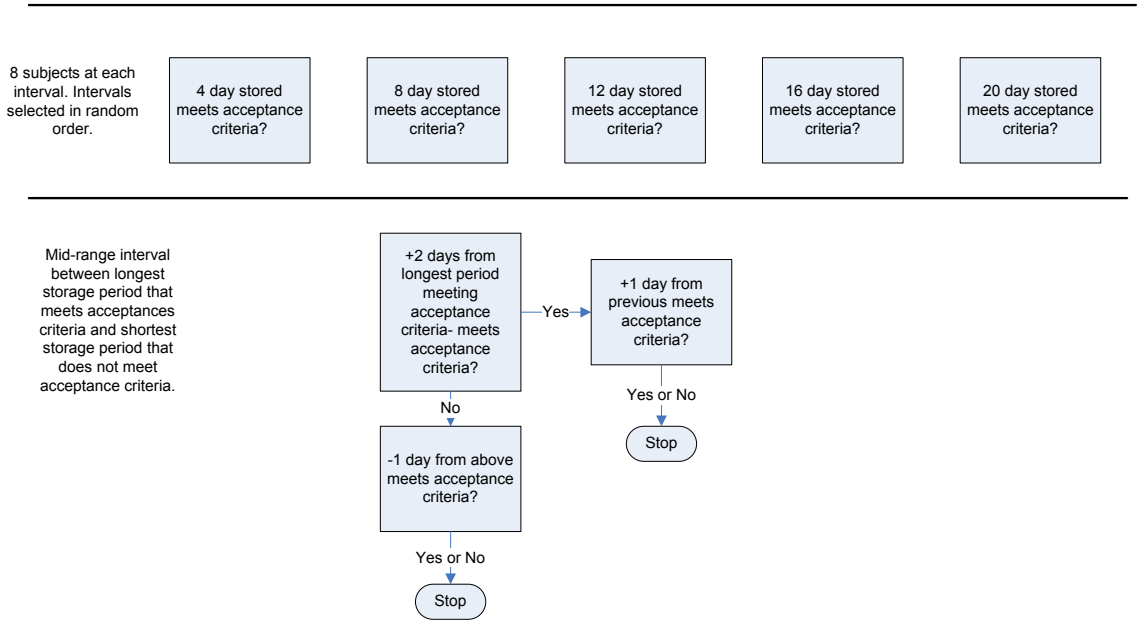

Figure 1: Study Schema

Subjects will donate one unit of whole blood. This unit will be maintained as whole blood in CPD (Citrate Phosphate Dextrose anticoagulant) not leukoreduced and not separated into components.

These WB units will be stored under refrigerated conditions (4°C) for between 4 and 20 days after collection. Prior to refrigeration samples from the unit will be obtained for various *in vitro* tests.

***In vivo* Measurements**

At the end of each subject's storage period a ~200 mL aliquot of whole blood will be withdrawn from the unit. This aliquot will be centrifuged and the platelets extracted. The

platelets will be radiolabeled with ^{51}Cr or ^{111}In. On that day, the subject will return to the Blood Center and provide a 43 mL fresh blood sample. Platelets from that sample will be harvested and labeled with the other isotope. The two isotopes will be rotated between the fresh and stored platelets so that at the end of the study equal numbers of fresh and stored platelets will have been labeled with each isotope. Radiolabeling will be done by the BEST method.[5] These aliquots will be sequentially infused into the subject. The estimated amount of the isotope is ≤15 μCi of indium and ≤20 μCi of chromium will be infused. The total radiation dose is approximately 40 μCi for a total body absorbed dose of 0.0273 rad (0.273 mSv) and a splenic absorbed dose of 3.2 rad (32 mSv). Venous samples (~10 mls/draw) will be taken within 2 hours after reinfusion and on days 1, 2, 3, 5, 7 ± 1, and 10 ± 2. After these visits, the subject's participation will end and they will exit the study. Stored and fresh platelet recoveries and survivals will be determined and comparisons will be made between each subject's fresh and stored platelet recoveries.

***In vitro* Assays:**

The following assays will be performed using a 50 mL sample obtained from the whole blood pre and post storage:

- Platelet Count. Platelet counts of the platelet products will be performed on an ABX Micros 60 (ABX, Montpellier, France) particle counter per manufacturer's instructions.

- Platelet Function Assays.
  - Mean Platelet Volume (MPV). MPV will be determined on the ABX Micros 60 (ABX, Montpellier, France) particle counter per manufacturer's instructions.
  - Morphology Score. Morphology score will be performed by the method of Kunicki.[6] A phase contrast microscope is used to determine the percentage of cells on a slide that are in the normal, discoid shape, are sphered, dendrites with long pseudopodia, or are balloons which are large spherical swollen platelets. A numerical score reflecting these cell types is calculated with the maximum score being 400.
  - Hypotonic Shock Response (HSR). HSR measures a platelet's ability to recover normal volume after the swelling induced by exposure to a hypotonic environment. Using a Chronolog 500-CA aggregometer (Havertown, PA), water is added to a curvette containing PRP. When platelets swell, their refractive index decreases, resulting in increased light transmission which is recorded photometrically. The ability to contract after the addition of the water is a measure of platelet function.[7]
  - Extent of Shape Change (ESC). During activation, platelets change from their typical discoid morphology to a more spherical shape. Platelet shape change is determined semi-quantitatively by light transmittance photometry using a Chronolog 500-CA aggregometer (Havertown, PA). The extent of shape change is determined by the increase in optical density of a platelet suspension prior to and after the addition of ADP in the presence of EDTA to prevent platelet aggregation.[7]

- Biochemical Status. A variety of biochemical assays including pH, PO2, PC02, HC03 will be measured using a Bayer 248 Blood Gas Analyzer (Bayer, East Walpole, MA).
- Glucose and lactate will be measured using an Abbott Aeroset Analyzer (Abbott, Round Lake, IL) at a local private laboratory (Dynacare, Inc.; Seattle, WA).

- Platelet Activation and Apoptotic Markers.
  - P-Selectin antigen (CD62). P-selectin (CD62) is an integral membrane protein associated with the α-granules of platelets. As platelets become activated, CD62 becomes expressed on the surface of platelets and is measured by a FITC-labeled S-12 antigen using flow cytometry.[8]
  - Annexin V. Annexin V is a protein that binds to phosphatidyl serine that becomes expressed on the platelet surface as the platelet ages. The amount of Annexin V expressed on platelets is determined by adding an Annexin V Alexa fluor to a platelet sample [Vybrant Assay Kit 1 (V-13240); Molecular Probes, Eugene, OR], and the amount of binding is determined by flow cytometry.
- Platelet Functional Tests
  - Platelet Aggregation - Measuring platelet aggregation response to standard agonists (ADP, collagen, arachidonic acid) by light transmission aggregometry (LTA) is an accepted method of detecting qualitative changes in platelet function. Using a Chrono- log 500CA, PRP will be exposed to standard concentrations of the agonists above, and changes in light transmission through the PRP suspension will be recorded as platelets aggregate.
  - Thrombelastograph (TEG) - This test provides a graphic representation of the fibrin polymerization process (clot formation) and may have implications for the stored platelet's ability to respond to conditions expected to exist at the site of vascular injury. This test will be performed at the University of Washington Hospital Laboratory.
  - Thrombin Generation Test - The calibrated automated thrombogram [CAT] system by Diagnostica Stago, Inc will be used to measure thrombin generation. In a previous study the velocity of thrombin generation and resistance against fibrinolysis were significantly reduced with increased storage time, suggesting a possible loss of hemostatic potential. This test may or may not be performed depending on instrument availability.
  - Platelet Microparticles - Cell membrane-bound particles of less than a micrometer in diameter, or microparticles, are biomarkers for cellular activation and/or apoptosis. They have been used as a potential marker of the platelet storage lesion. We will be using a BD FACSCalibur flow cytometer and the procedure developed by Larry J. Dumont, MBA, PhD, Director, Cell Labeling Lab, Dartmouth-Hitchcock Medical Center.

Plasma Analysis of Coagulation, Complement and Microparticles

- US Army Institute of Surgical Research (ISR) will measure Thrombin-Antithrombin complex (TAT - Enzygnost TAT micro, Siemens Healthcare) as a marker of activation of the coagulation system, plasmin-antiplasmin complex as a marker of activation of the fibrinolytic system (PAP - Imuclone PAP ELISA, Ameircan Diagnostica), and soluble CD40L as a measure of platelet activation and granule release (sCD40L - Human sCD40L Platinum ELISA Extra Sensitive, eBioscience).
- ISR will measure the level of plasminogen activator inhibitor (PAI-1 - Zymutest PAI-1 Antigen ELiSA, Aniara) as a secondary index of fibrinolytic potential.
- ISR will measure the following markers of complement system activation: C3a - Microvue C3a Plus EIA Kit, quidel corporation C4d - Microvue C4d EIA Kit, quidel corporation C5a - Microvue C5a EIA Kit, quidel corporation, C5b-9 - Microvue SC5b-9a Plus EIA Kit, quidel corporation.
- Platelet Microparticles - Cell membrane-derived particles of less than a micrometer in diameter, or microparticles, are biomarkers for cellular activation and/or apoptosis. They have been used as a potential marker of the platelet storage lesion. We will be using a BD FACS Canto II flow cytometer equipped with a forward scatter PMT that provides a better resolution of the signal from the noise in the lower particle size range and a labeling procedure developed at the US Army Institute of Surgical Research.

- Sterility
  - Bacterial cultures will be submitted on the day after collection and the results evaluated 14 days later, or sooner if blood is stored for a shorter time period. A Gram stain will be performed on the isolated 4° C platelets before reinfusion at the end of storage. All sterility tests must be negative before test platelets will be infused. Bacterial culture and Gram stain samples will be sent to the University of Washington Microbiology Laboratory for testing.

10 INCLUSION / EXCLUSION CRITERIA

10.1 SUBJECT ELIGIBILITY

A subject will be considered eligible if at the time of enrollment it is determined that ALL of the inclusion criteria and NONE of the exclusion criteria are met. Subjects who are enrolled in good faith after having undergone the screening and enrollment process, but for whom later are learned to have not satisfied all of the inclusion and exclusion criteria (e.g., after reinfusion of the radiolabeled cells, a subject calls and discloses that she will be travelling outside the country for the next 2 weeks, etc.) will be considered enrolled, but not evaluable for the primary endpoint. Their results will not be included in

the primary endpoint analysis. Enrollment will continue until the required numbers of evaluable subjects are accrued in the study.

10.2 INCLUSION CRITERIA

The study will enroll up to 80 healthy subjects that meet the following inclusion criteria:

1. The subject is in good health, is taking no excluded medications, and satisfies the Blood Center's criteria to donate blood products (see attached PSBC Donor History Questionnaire). Criteria will also include the following guidelines:
    - Meets whole blood/platelet donor suitability requirements as defined in 21 CFR 640.3 and set forth by the AABB (Reference Standard 5.4.1A, 2006). Past travel restrictions do not apply for this study.
    - Weight ≥50 kg (110 pounds)
    - Hemoglobin/hematocrit: ≥12.5 g/dL/≥38%
    - Temperature: ≤37.5°C
    - Resting blood pressure: systolic ≤ 180 mmHg; diastolic ≤100 mmHg
    - Resting heart rate: 50 to 100 beats per minute
2. Age ≥ 18 years, of either sex;
3. Able to read, understand and sign the informed consent document and commit to the study follow-up schedule;
4. Subjects must have good veins for whole blood collection and follow-up blood draws;
5. Subjects of child-bearing potential must agree to use an effective contraceptive during the course of the study;
6. Those subjects who will be reinfused within 10 days of their 500 mL WB collection must meet the criteria for donating a double RBC unit to accommodate the sampling volume that will occur shortly thereafter:
    a. Male subjects must weigh a minimum of 130 lbs and be at least 5'1" tall
    b. Female subjects must weigh a minimum of 150 lbs and be at least 5'5" tall
    c. Both male and female subjects must have a Hematocrit of ≥ 40%;

10.3 EXCLUSION CRITERIA

Healthy subjects will be excluded from the study for any of the following reasons:

1. Any serious medical illness and/or therapy, including: abnormal bleeding episodes, clotting or bleeding disorder, evidence of anemia, myocardial infarction, uncontrolled hypertension, heart disease, surgery with bleeding

complications, epilepsy or any major surgery (with general or spinal anesthesia) within the last 6 months;

2. Taking aspirin, Alka-Seltzer™, clopidrogel, or other "anti-platelet" drugs within 7 days prior to donation.

3. Taking any nonsteroidal anti-inflammatory drug (for example: Motrin™, Advil™, or ibuprofen) within 3 days prior to donation.

4. Taking any anticoagulant medications (for example Coumadin, dabigatran, rivaroxaban or any medications chemically related to heparin).

5. Currently pregnant or nursing within the 6 weeks prior to enrollment as assessed during interview. Current status is confirmed by pregnancy test prior to radioisotope infusion.

6. Inability to comply with the protocol in the opinion of the investigator.

7. Participation in another investigational trial that would potentially interfere with the analysis of this investigation (e.g., pharmaceutical);

8. Have participated in ≥ 4 research studies involving radioisotopes within the last 12 months.

11 STUDY PROCEDURES

11.1 OVERVIEW OF STUDY SCHEDULE

Figure 2, on the following page, shows the flow of subjects and data collection throughout the study. The top section shows the subjects' initial involvement in the study, as they go through the informed consent process, are screened, enrolled, and then donate a unit of WB. The middle section shows *in vitro* testing of the platelets derived from refrigerated whole blood throughout the storage period. Detailed descriptions of the procedures can be found in the following sections and in the additional referenced study materials.

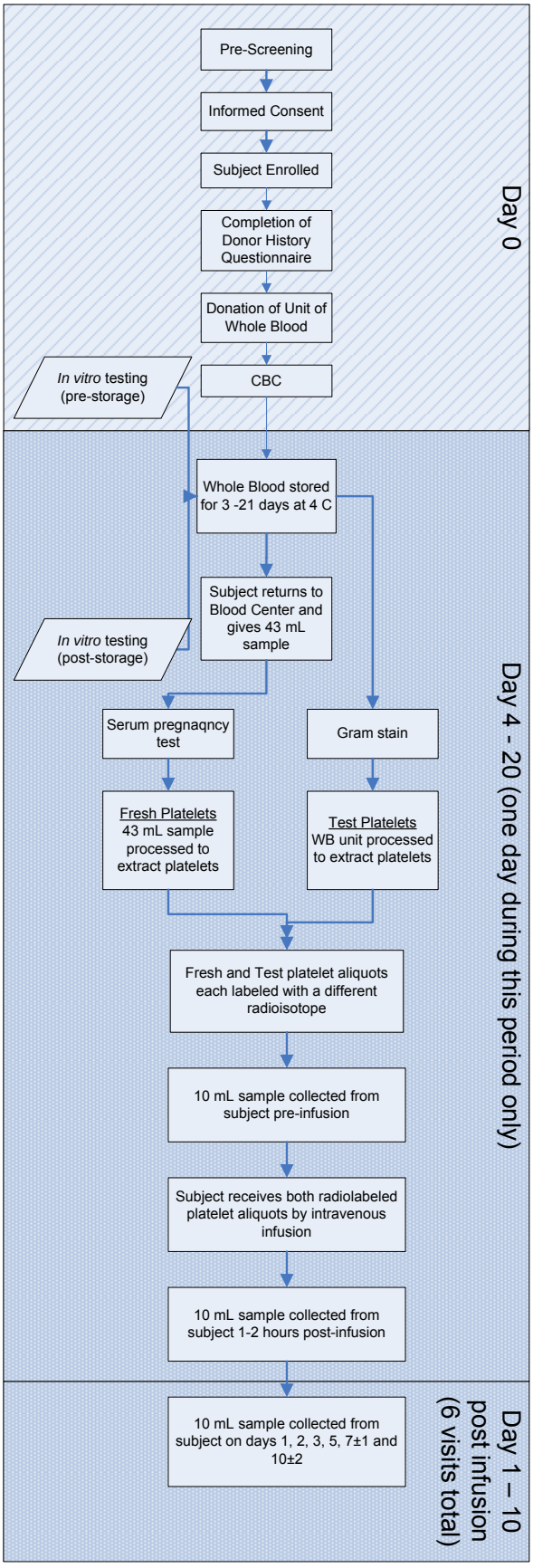
Day 0
Pre-Screening
Informed Consent
Subject Enrolled
Completion of Donor History Questionnaire
Donation of Unit of Whole Blood
CBC
In vitro testing (pre-storage)
Day 4 - 20 (one day during this period only)
Whole Blood stored for 3 -21 days at 4 C
Subject returns to Blood Center and gives 43 mL sample
In vitro testing (post-storage)
Serum pregnaqncy test
Gram stain
Fresh Platelets 43 mL sample processed to extract platelets
Test Platelets WB unit processed to extract platelets
Fresh and Test platelet aliquots each labeled with a different radioisotope
10 mL sample collected from subject pre-infusion
Subject receives both radiolabeled platelet aliquots by intravenous infusion
10 mL sample collected from subject 1-2 hours post-infusion
Day 1 – 10 post infusion (6 visits total)
10 mL sample collected from subject on days 1, 2, 3, 5, 7±1 and 10±2

Figure 2: Flow of Study Procedures

11.2 SCREENING, CONSENT AND BLOOD COLLECTION

Healthy adults responding to a posting on the University of Washington's website will come to the Puget Sound Blood Center to be consented, screened and undergo whole blood collection. Standard Blood Center procedures, used for community blood donors, will be performed. Donors are screened for disease risk factors using a health history questionnaire. Donors are asked specific and direct questions regarding lifestyle, health, medical history and travel to assure their own health will not be compromised by a blood donation and that patients receive safe blood products. Donors can be deferred for a variety of reasons: signs and symptoms of relevant transfusion-transmitted infections, such as HIV, viral hepatitis, HTLV, syphilis or West Nile virus; social behaviors that increase their risk of exposure to infectious diseases, including men who have sex with other men, intravenous drug use and exchanging sex for drugs or money; travel to certain countries where the risk of exposure to a particular infectious disease is of concern; medical procedures that involve receipt of dura mater graft, transfusion of blood or blood components within the previous 12 months, or human-derived clotting factors within the previous 12 months; incarceration under certain circumstances; obtaining a piercing or tattoo using nonsterile materials within the previous 12 months; certain medications; and pregnancy.

Additional requirements include:

1. Weight: 50 kg (110 pounds).
2. Hematocrit: 38%
3. Oral Temperature: 37.5C
4. Blood Pressure: Systolic 180 mmHg; diastolic 100 mmHg
5. Heart Rate: 50 to 100 beats per minute

These vital sign requirements are the same as those in the protocol.

For participation in this study no infectious disease testing will be done and travel restrictions do not apply

The schedule of events is given in Table 1, on the next page.

Table 1. Schedule of Events During Study

Study Day	Study Procedures
Day -4 to -20	Informed consent process
	Screening and enrollment
	Blood donation
	In vitro testing
	Bacterial culture sample collected from WB unit (day after collection)
Reinfusion day **Day 0**	Whole blood storage ends
	Aliquot removed from stored WB
	Post-storage *in vitro* testing
	Bacterial cultures evaluated
	Gram stain on stored unit
	43 ml blood sample from subject processed to extract fresh platelet aliquot
	If subject is pre-menopausal female, pregnancy test completed
	Fresh and test platelets radiolabeled
	Subject and samples confirmed for matching ID
	Reinfusion of radiolabeled fresh and test platelets
	Post infusion samples drawn (1-2 hours post-infusion)
Post infusion **Day 1**	1 day sample drawn
Post infusion **Day 2**	2 day sample drawn
Post infusion **Day 3**	3 day sample drawn
Post infusion **Day 5**	5 day sample drawn
Post infusion **Day 7(± 1 day)**	7 day sample drawn
Post infusion **Day 10(± 2 days)**	10 day sample drawn
	Subject exits study

11.3 SPECIFIC STUDY PROCEDURES

11.3.1 TOTAL VOLUME OF WHOLE BLOOD COLLECTED

After a subject has completed the informed consent process and has been enrolled in the study, standard Puget Sound Blood Center procedures, used for community blood donors, will be performed including; completion of a Donor History Questionnaire, review of medications, documentation of the subject's vital signs and collection of one unit of WB. Approximately 500 mL ± 10% of WB will be drawn in CPD anticoagulant from each subject. A 2 mL CBC sample will be collected from the in-line sampling pouch. No infectious disease testing will be done. Several days later, on the day the subject receives

his or her autologous platelet infusion, 43 mL will be drawn, to make the fresh platelet aliquot and an immediate pre-infusion sample of 10 mL for evaluation of baseline radioactivity will also be drawn. A 3 mL pregnancy sample will also be collected for pre-menopausal women. One hour after infusion a 10 mL post-infusion sample will be collected for radioactivity evaluation. In the post reinfusion evaluation, follow-up blood draw volume will total approximately 60 mL (10 ml x 6 samples) over the course of their 10 days of follow-up. Total volume of blood drawn from subjects will be approximately 628 mL.

Table 2. Schedule of Blood Draw Volumes

	CBC (from in-line sampling pouch) (2 mL)	Blood Donation (500 mL ± 10% WB)	Serum Pregnancy (3 mL)	Sample to derive 'fresh' platelet aliquot (43 mL)	Radioactivity sample (10 mL)
Enrollment and WB Collection (Day -4 to -20)	√	√			
Pre Reinfusion Day 0			√	√	√
Post Reinfusion Day 0					√
Day 1					√
Day 2					√
Day 3					√
Day 5					√
Day 7 (± 1 day)					√
Day 10 (± 2 day)					√
Total mLs	2	500	3	43	80

11.3.2 SUBJECT DONATION OF WHOLE BLOOD UNIT

The unit of WB that is donated by each enrolled subject will be collected in accordance with standard protocols including the use of universal precautions when handling blood products. Blood will be prepared and stored at the Puget Sound Blood Center. All collections will be made in a FDA licensed whole blood collection bag with CPD anticoagulant and will not be leukoreduced.

11.3.3 *IN VITRO* TESTING SCHEDULE

Table 3, on the following page, provides a listing of the tests to be performed on the WB units at the beginning and end of storage. The unit volume, platelet count, platelet yield, mean platelet volume and white blood cell count will be calculated off the WB unit. The

blood gases, platelet assays, TGT, platelet aggregation, glucose and lactate will be performed on a PRP aliquot made from the WB unit.

Table 3. ***In vitro*** **Tests Performed on Test Units**

Test Type	Time Point for Cell Quality Testing of Platelets derived from Whole Blood (WB)	
	WB collection day	End of storage
Plt count	✓	✓
Mean Platelet Volume	✓	✓
Morphology Score	✓	✓
Hypotonic Shock Response	✓	✓
Extent of Shape Change	✓	✓
Blood Gases (pH and pCO2, PO2, HC03)	✓	✓
Glucose/Lactate	✓	✓
Annexin V	✓	✓
P-Selectin Antigen (CD62)	✓	✓
Platelet Aggregation	✓	✓
Thrombelastograph (TEG)	✓	✓
Thrombin Generation Test (TGT)	✓	✓
Platelet Microparticles	✓	✓
Plasma analysis of Coagulation Factors, Complement and Microparticles ☸	✓	✓
Bacterial Culture	✓*	
Gram stain		✓

All samples will be discarded at the end of the study.

☸Testing done on frozen aliquots shipped to Blood Research Program, US Army Institute of Surgical Research

*Bacterial Culture sample removed from unit on day after collection and evaluated at end of storage.

11.3.4 DONOR WHOLE BLOOD (UNTREATED IN CPD)

After the blood unit is donated and labeled with the subject's unique ID, an aliquot of blood will be removed from the unit and tested per Table 3 above.

11.3.5 STORAGE PROCEDURE FOR WHOLE BLOOD

The original 500 mL collection bag will be placed immediately (< 8 hours after collection) in a temperature-monitored and controlled environment (Bally® walk-in cooler with a REES monitoring system) at 4 ± 2 °C, without agitation. Temperature monitors will record changing temperatures during the storage period.

End of storage will be defined as the date and time when the aliquot for radiolabeling and reinfusion is removed from the stored unit.

11.3.6 CONFIRMATION OF SUBJECT HEALTH STATUS AND ID

Prior to infusion of radiolabeled, autologous platelets into the subject, the subject's health will be re-assessed via interview.. If the subject feels unwell, has cold- or flu-like symptoms, or has any negative change to his or her health status, then he/she will be considered ineligible for the radiolabel reinfusion and will exit the study.

Pre-menopausal female subjects will have a serum pregnancy test to confirm that they are not pregnant prior to reinfusion. All subjects with a positive pregnancy test will be ineligible to continue with the reinfusion and will exit the study.

Two members of the study team will confirm that the correct sample is taken from the correct stored WB unit for the matching subject, by verification and documentation of the correct subject information labeling.

11.3.7 *IN VITRO* TESTING: END OF STORAGE

At the end of the storage period an aliquot of approximately 50 mL of the stored Whole Blood unit will be used for *in vitro* testing. Table 3 provides the *in vitro* parameters for evaluation at this time point.

11.3.8 RECOVERY AND SURVIVAL PROCEDURES

At the end of the storage period, after the *in vitro* testing sample has been removed from the unit, a 50 mL aliquot of Whole Blood will be removed via a sterile connecting device, centrifuged at room temperature and the platelets separated for radiolabeling.

The platelet fraction will be radiolabeled with ^{51}Chromium or ^{111}Indium using the platelet research unit's standard operating procedure. In brief, the platelets obtained from the WB will be labeled by adding ^{51}Cr or ^{111}In at the appropriate activity level and incubated with mixing at room temperature for 30 minutes. The platelets will be washed in saline to remove unbound ^{51}Cr or ^{111}In in the supernatant and the platelets will be resuspended in saline.

Infusion of the radiolabeled fresh and stored platelets will be conducted according to the platelet research unit's standard operating procedure. After a good venous line has been established, a blood sample will be obtained to check baseline radioactivity. Subject information and radioactivity level of infusion will be verified prior to infusion of the radioactive platelets. Approximately 10-12 mL of the fresh and stored radiolabeled platelets will be autologously infused back into the subject. After infusion, the line will be flushed with saline and removed. The subject will remain at the Blood Center or return to the Blood Center for the post-infusion, Day 0, blood samples, which will be collected 1-2 hours after the infusion.

Samples (approximately 10 mL) will be drawn during follow-up visits on Days 1, 2, 3, 5, 7 ± 1 and 10 ± 2 to calculate the platelet survival.

11.3.9 TRANSPORTATION OF SPECIMENS

Samples for bacterial culture, Gram staining and TEG will be transported to the University of Washington by Puget Sound Blood Center courier or taxi, in a sealed, puncture proof container, labeled as biohazard. This is also the method we will use to convey samples for glucose and lactate testing to LabCorp. This method is currently utilized by the Puget Sound Blood Center as standard practice. Samples for coagulation complement and microparticle analysis will be sent to the Blood Research Program, US Army Institute of Surgical Research (ISR), Fort Sam Houston, Texas by overnight courier (such as Fed Ex or UPS).

11.4 SUBJECT COMPENSATION

Subjects will receive $300.00 at the conclusion of the study for their time involved in study participation. If the subject is unable to complete the study or has to be withdrawn from the study, they will receive partial payment for their time involved in the study. The partial payment scale is the following (number in parentheses equals the number of times each procedure occurs during the course of the study):

Initial screening (Visit 1a)		$25
Blood collection (Visit 1b)		$50
Collection of 43 mL of whole blood (Visit 2)		$25
Reinfusion of fresh and stored platelets (Visit 3)		$25
First follow-up blood sample 1- 2 hours post platelet infusion (Visit 4)		$25
Follow up blood draws (Visits 5-10) $25 each		$150
Total for completing all study procedures	=	$300

12 ADVERSE EVENT (AE) ASSESSMENTS

During each reinfusion of platelets, the subject will be carefully monitored for adverse reactions; e.g., fever, chills, dyspnea, urticaria, or pain (infusion site, chest pain or other). Adverse reactions will be recorded in the study file and reported to the study investigator.

Subjects will be instructed to report changes in health condition over the course of the study to the study coordinators. Minor AEs that are associated with venipuncture and blood donation, such as minor bruising at the needle site, will not be recorded as AEs, unless they worsen over time (e.g., become infected, etc.).

13 DATA ANALYSIS / STATISTICAL METHODS

Summary statistics (means, standard deviations, confidence limits) will be calculated for all in-vitro assays and in-vivo platelet function measures: recovery and survival. A table of *invitro* summary statistics will be presented to facilitate comparison of assay values from the day of WB collection and the end of WB storage sample.

Tables of recovery and survival summary statistics will display values from fresh and stored platelets. Recovery and survival means and means of stored recovery – 0.50 x fresh recovery will be plotted against days stored. Regression methods will be used to determine if there are any trends in the means with respect to time. Joint lower 95% confidence limits for recovery difference and survival will be calculated to determine storage intervals for which the WB stored platelets meet performance criteria.

14 DATA MANAGEMENT

14.1 REQUIRED DATA

For the duration of the study, the investigator will maintain complete and accurate documentation, including but not limited to study progress notes, laboratory reports, signed subject informed consent forms, correspondence with the reviewing IRB, and sponsor, adverse event reports, and information regarding subject discontinuation or completion of the study.

The investigator/institution will permit direct access to source data and documents in order for study-related monitoring, audits, IRB reviews, event adjudication and regulatory inspections to be performed. The investigator will obtain, as part of the informed consent process, permission for sponsor, authorized sponsor employees or representatives, representatives of the U.S. Army Medical Research and Materiel Command (USAMRMC), study monitors or regulatory authorities to review, in confidence, records that identify subjects in this trial.

Accurate and complete study records will be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research.

14.2 CONFIDENTIALITY

The sponsor will consider all data and information collected during this study confidential. All data used in the analysis and summary of this study will be anonymous, and without reference to specific subject names. Study records, samples, and test results will be identified with a unique identifier and access will be limited to sponsor authorized personnel, the investigator, site research staff, and authorized regulatory authorities, including representatives of the FDA. Study files will be kept in a locked area with

restricted access. Disposal of subject samples will be conducted according to the blood center's standard procedures for biohazard disposal. Screening of subjects will be conducted by trained members of the research team in an area that provides visual and auditory privacy according to the blood center's standard procedures.

15 RISKS / BENEFITS ASSESSMENT

15.1 RISKS OF VENIPUNCTURE AND RADIOLABEL INFUSION

With each venipuncture performed to collect a blood sample there may be some extravasation of blood into the tissues during the venipuncture causing a hematoma. Other common, mild side effects are: stinging or pain during insertion of the needle, upset stomach or vomiting, dizziness, thirst, sweating, rapid pulse, or fainting.

More serious, but rare complications like nerve injury may occur if during the venipuncture the needle damages a nerve. Also, if the skin is not adequately cleaned before the venipuncture there may be a risk of infection at the venipuncture site. Other serious side effects may include: hives, bronchospasm, allergic reactions, muscle spasms or cramps, convulsions, or vessel damage (including dissection or puncture).

Since subjects will be transfused with their own platelets the risk of a transfusion reaction is minimal, however, in very rare cases, a reaction may occur, causing severe illness or death. A registered nurse will closely monitor the subject during the reinfusion of their platelets.

The amount of radiation exposure received by the subject is low and is not considered to be harmful. However, women who are pregnant or who are nursing will be excluded from the study, as the risks of radiation exposure to a fetus or infant are unknown. All pre-menopausal women will have a pregnancy test performed and prior to reinfusion. Any subject with a positive pregnancy test will not be given radiolabeled platelets and will be withdrawn from the study.

There is a possibility of bacterial overgrowth if bacteria have inadvertently entered the blood bag collection system. However, a sample of the stored Whole Blood will be cultured using a commercially available test. In addition, a Gram stain to detect bacteria will be performed on the stored platelets on the day of reinfusion. The results of these tests will be available prior to the infusion of cells. Any subject with positive results will not be re-infused with their platelets.

The maximum volume of blood collected during the course of this study, over 14-30 days, is approximately 628 mL. The standard blood center eligibility criteria for donating blood products includes tracking RBC loss. Over a 12-month period a donor is allowed a total of no more than 1477 ml of RBC loss. A standard single unit WB collection (211 ml of RBC loss) is between 468-558 ml and a subject can donate 1 unit of WB every 56 days. After a WB collection the body replaces the plasma in about 24 hours, RBCs are restored in two to four weeks, and platelets are replenished in about 72 hours. To ensure the subject's safety, eligibility for subjects who will have sample draws within 10 days of their WB collection will be restricted to those volunteers who qualify for a double RBC

donation, and the donation interval of 112 days (deferral period for a double RBC unit donation) after his/her exit from the study will be placed on the subject's donation record to prevent a subject from donating a blood product before they are eligible.

15.2 MINIMIZATION OF RISKS

Risks for the following study interventions will be minimized as described.

Collection of a unit of whole blood

Blood donation includes a check of vital signs, hematocrit and assessment that the donor-subject is in general good health. Blood Center personnel are trained in the proper methods of venepuncture to reduce discomfort and complications. Staff are also trained and experienced in management of donation reactions. Subjects will be instructed to report any local signs or symptoms that suggest infection or phlebitis at a venepuncture site.

Re-infusion of platelets

Study staff and technical personnel will employ standard American Association of Blood Bank procedures to avoid contamination of platelets during processing and handling. Microbial cultures and Gram stain will be performed prior to reinfusion of platelets to monitor for contamination. Only units for which culture and Gram stain are negative will be re-infused.

Since subjects will be transfused with their own platelets, the risk of a transfusion reaction is minimal. Several steps will be taken to prevent misidentification. First, the blood bag holding the subject's whole blood will be tagged with the subject's name, date of birth, a unique blood unit number and a study identification (ID) number. The identifiers on this tie tag will be carried through on all containers used in the labeling procedure. In addition, if more than one person's blood will be undergoing labeling at the same time in the laboratory; all transfers of blood will be verified by two technologists to minimize the possibility of a sample mix-up. The subject's identifiers will be cross checked against those on the tie tags by two staff members prior to reinfusion of any and all blood components.

A Registered Nurse will closely monitor the subject during the reinfusion of their platelets.

The Puget Sound Blood Center is located within several blocks of three major medical centers and has very ready access to emergency medical care. Blood unit collection will be performed by registered nurses that are CPR certified and trained in emergency medical procedures. An on-call MD can always be summoned by pager. Follow-up blood sample draws and product infusion will be conducted at the Blood Center by experienced staff that is CPR certified and trained in emergency medical procedures. Registered Nurses will monitor subjects throughout the blood collection and platelet infusion. Should significant symptoms arise staff will stop the collection/infusion, keep IV access open, perform clerical checks as indicated, summon the PI or physician on call to obtain further orders to address subject symptoms, and be prepared to institute emergency measures as necessary.

Radioactivity

The amount of radiation exposure received by the subject is low and is not considered to be harmful. If the subject is sexually active, they are instructed to use a method to prevent pregnancy during the course of the study. This applies to both men and women. The risks of radiation exposure to a fetus are unknown. Therefore, women of childbearing potential will have a pregnancy test performed prior to the radiolabeled platelet reinfusion. Any subject with a positive pregnancy test will not be given radioactively labeled platelets and will be withdrawn from the study.

The Blood Center's Platelet Transfusion Research Department will maintain a record of subject participation in this study, and the total radiation exposure in any 12-month period will be kept below limits imposed by general guidelines (≤5 rad or ≤0.05 Sv splenic dose). Subjects will be instructed to inform investigators of any other radiation exposure (such as for diagnostic tests) so that those doses are included in the cumulative annual dose for each subject.

15.3 POTENTIAL BENEFITS

Besides a nominal payment for participation in this study, there is no direct benefit to the study subject. Real benefits are altruistic in nature: subjects participating in this study will assist the scientific and medical communities in gathering important information to assess novel procedures for improving the availability of platelet transfusions.

16 ADVERSE EVENT REPORTING AND OVERSIGHT

16.1.1 ADVERSE EVENTS

An AE is any undesirable event that is associated with the study procedures and does not depend on the causal relationship with the device or protocol requirements. An event may be evidenced by clinical signs or symptoms, abnormal results of clinical laboratory or other safety tests (e.g., ECG, chest x-ray), development or exacerbation of intercurrent illness. AE data will be reported as follows:

- Expected AEs will be reported to the investigator,
- Documentation of the AE will be performed by the reporting individual and signed by the contacted physician.
- A copy of this event will be placed in the subject's study file. These events will be reviewed and signed by the investigator.
- Any expected adverse event will be reported to the IRB at the time of renewal or sooner if there is a greater frequency, severity or other findings of note per the institution's IRB requirements.
- The study monitor will be notified of adverse events.

Pre-existing conditions will not be reported as an adverse event unless there has been a worsening in the severity or frequency, which cannot be attributed to natural progression

of the condition. Minor AEs that are associated with venipuncture and blood donation, such as minor bruising at the needle site, will not be recorded as AEs, unless they worsen over time (e.g., become infected, etc.)

16.1.2 SERIOUS ADVERSE EVENT DEFINITION

A Serious Adverse Event (SAE) is one which suggests a significant hazard, contraindication, side effect or precaution. An adverse event is considered serious if the event:

- Leads to death;
- Leads to a serious deterioration in the health of the subject that:
  - Results in life-threatening illness or injury;
  - Results in permanent impairment of a body structure or a body function;
  - Requires inpatient hospitalization or prolongation of existing hospitalization;
  - Results in medical or surgical intervention to prevent permanent impairment to a body structure or a body function.

When an adverse event or complication meets the definition for an SAE, it will be considered as such and reported immediately upon knowledge to the sponsor and to the reviewing IRB per institutional requirements.

SAEs will be reported as follows:

- Unexpected or Serious Adverse Events will be reported as soon as possible to the investigator or designee, either in person or by phone.
- Documentation of the SAE will be performed by the reporting individual and signed by the contacted physician. A copy of this event will be placed in the subject's study file. The PI and IRB will be notified as soon as possible of this SAE and according to the IRB's guidelines.
- An Adverse Event CRF will be completed and sent for review.
- The sponsor will be notified immediately in the event of an SAE.

16.1.3 UNANTICIPATED ADVERSE DEVICE EFFECTS DEFINITION

Unanticipated adverse device effects (UADE) are defined as any serious adverse effect on health, safety or any life-threatening problem or death caused by, or associated with, the investigational device; if that effect, problem, or death was not previously identified in nature, severity or degree of incidence in the study protocol and informed consent. When an adverse event meets the definition of an UADE or that relationship is unknown, the investigator will report the event to the sponsor immediately after the investigator first learns of the effect and to the reviewing IRB as required.

Reporting steps for events meeting the description of an UADE should be handled in the same manner as those meeting the definition of an SAE.

16.2 SAFETY MONITORING

Reports for events determined by the investigator to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the study sponsor, the Office of Research Protections, Human Research Protection Office (ORP, HRPO), and the Puget Sound Blood Center's local IRB.

Data Safety Monitoring Board: There will not be a Data Safety Monitoring Board for this study because it involves healthy subjects undergoing a commonly practiced research evaluation procedure.

16.3 USAMRMC REPORTING REQUIREMENTS FOR SAE AND UADE

All unanticipated problems involving risk to subjects or others will be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the Human Research Protection Office (HRPO). A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

17 RESEARCH MONITOR

The Research Monitor, Terry B. Gernheimer, MD, Director - Medical Education at the Puget Sound Blood Center, and Professor of Medicine and Director of Medical Transfusion Services at the University of Washington is responsible to oversee the safety of the research and report observations/findings to the University of Washington's Human Subjects Division, which is the IRB of Record. Dr. Gernsheimer will review all unanticipated problems involving risk to volunteers or others associated with the protocol and provide an unbiased written report of the event to the UW's IRB. Dr. Gernsheimer may discuss the research protocol with the investigator, interview human subjects, and consult with others outside of the study about the research. Dr. Gernsheimer shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects.

18 USE OF INFORMATION AND PUBLICATION

All information and data generated in association with this study will be held in strict confidence and remains the joint property of the Puget Sound Blood Center and the USAISR Coagulation and Blood Research Task Area. This collaborative effort is envisioned to lead to development of new platelet storage techniques. Joint authorship in publications and inventors rights will be shared by both parties.

19 REFERENCES

[1] Slichter, SJ, Harker LA. Preparation and storage of platelet concentrates. II. Storage variables influencing platelet viability and function. Br J Haematol 1976; 34(3):403-419.

[2] Defense Health Board Memorandum of June 2, 2008. DHB 2007-04 2008.

[3] Borgman MA, Spinella PC, Perkins JG et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma 2007; 63:805-813.

[4] Hess JR, Holcomb, JB. Transfusion practice in military trauma. Transfusion Med 2008; 18:143-150.

[5] The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Transfusion:46:59S-66S.

[6] Kunicki TJ, Tuccelli M, Becker GA, Aster RH. A study of variables affecting the quality of platelets stored at room temperature. Transfusion 1975;15:414-21.

[7] Holme S, Moroff G, Murphy S. A multi-laboratory evaluation of *in vitro* platelet assays: the tests for extent of shape change and response to hypotonic shock. Biomedical Excellence for Safer Transfusion Working Party of the International Society of Blood Transfusion. Transfusion 1998;38:31-40.

[8] Dumont LJ, VandenBroeke T, Ault KA. Platelet surface P-selectin measurements in platelet preparations: an international collaborative study. Trans Med Rev 1999;13: 31-42.

UNIVERSITY OF WASHINGTON
CONSENT FORM
Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

Researchers: Sherrill J. Slichter, M.D., Director of Platelet Transfusion Research, Puget Sound Blood Center, and Professor of Medicine, University of Washington School of Medicine; (206) 292-6541

Jill Corson, R.N., Research Nurse, Puget Sound Blood Center, (206) 292-6392

Lynda Fitzpatrick, R.N., Research Nurse, Puget Sound Blood Center, (206) 292-2347

MeLinh Jones, BS, Clinical Research Coordinator, Puget Sound Blood Center, (206) 292-2535

Mary Kay Jones, MT(ASCP), Research Technologist, Puget Sound Blood Center, (206) 292-1871

Study Sponsor: Department of Defense (DOD), Telemedicine & Advanced Technology Research Center (TATRC), U.S. Army Medical Research and Materiel Command (USAMRMC)

24-hour emergency telephone number and study contact:

Call: Sherrill J. Slichter, M.D. - 206-292-6525

Researchers' statement

We are asking you to be in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called "informed consent." We will give you a copy of this form for your records.

PURPOSE OF THE STUDY

Platelets are a blood cell. Platelets are collected from volunteer blood donors. They are given to patients to treat and prevent bleeding. They can be critical in saving lives following trauma by helping blood to clot. Donated platelets are usually stored at room temperature. Because bacteria can grow in platelets stored at room temperature, the FDA has restricted their shelf-life to 5 days. As a result of this short storage time, some donated platelets are thrown-out before they can be used. This increases platelet shortages. We need to develop other methods of storing platelets to increase their storage time. This is especially important for the military. In combat situations platelets are often not available near the point of injury where they are most needed.

Our study plans to look at the function of platelets stored in the refrigerator, within the donated whole blood unit. We think that refrigerated platelets might stop bleeding in the short term. The

transfusion of cold stored platelets could help stabilize trauma patients before they are moved to a fully equipped hospital.

The purpose of our study is to measure the life span and quality of platelets stored in whole blood in a refrigerator for up to 20 days. We want to know if at least half of the platelets survive for at least one day following transfusion.

STUDY PROCEDURES

If you choose to take part in this study we will collect a pint (unit) of blood from you. The unit will be stored in the refrigerator. At the end of the storage period we will remove some blood from the unit and separate out the platelets. The platelets will be combined with a chemical element that emits radiation, also known as a radioactive isotope. You will return to the Blood Center on the day your blood is taken out of refrigerator storage. On that day we will collect a fresh blood sample from you. Platelets from your fresh sample will be combined with another, radioactive isotope. These isotopes stick to the platelets. Platelets treated this way are called radiolabeled platelets. This is how we mark or tag the platelets. We will inject you with both your stored and fresh radiolabeled platelets. After the injection we will draw samples from you to measure the levels of these radioactive isotopes in your blood. This will give us information on how many transfused platelets survived the refrigeration and are still in your blood stream. This information will be compared to the survival of your fresh platelets. Below is a more detailed description of what the study involves.

Study Visit 1

Consent and Eligibility Screening

Your first study visit will take about 60 minutes. You will be asked to read this consent form. After all your questions have been answered, if you wish to be in this study, you will need to sign this form. You will be asked to fill out a Donor History Questionnaire which includes questions about your health history and behaviors, including sexual activity, and medications that you have taken.

You will not be eligible to participate in this study if you have recently taken, or intend in the near future to take any of the following medications:

- Aspirin or aspirin-containing drugs (e.g., Alka-Seltzer) known to affect platelet function
- Plavix (clopidrogel bisulfate)
- Ticlid (ticlopidine hydrochloride)
- Non-steroidal anti-inflammatory drug (NSAID) such as ibuprofen or naproxen.
- Anticoagulant medications (for example Coumadin, dabigatran, rivaroxaban, apixaban or any medications chemically related to heparin)

If you need to take a pain reliever or fever reducer while in this study you can take Tylenol®/acetaminophen. You will be asked to report all medications you are taking while in this study.

A brief physical exam will be performed. We will record your height, weight, temperature, blood pressure and pulse.

If you have previously been registered in the Puget Sound Blood Center's *Donor Registration System* results of prior visits will be reviewed. If you do not qualify at this time, you may be able to return at a later date for screening.

You will be told that, while in this study, both men and women who are able to have children should either stop having sex or practice birth control.

During this visit, we will tell you how long your blood will be stored and when you need to return to the Blood Center.

Blood Collection

The skin on the inside of your arm will be cleaned with an antiseptic and a needle will be inserted through the skin into a vein. Blood will drain by gravity through the needle via plastic tubing into a plastic storage bag. The collection set is a closed sterile system. A blood pressure cuff may be wrapped around your upper arm to increase the rate of blood flow into the bag. You may be asked to hold and squeeze an object to increase the flow rate. Collection of a pint of blood (500 mL) usually takes 10 -15 minutes. After the donation is over, the needle is removed and the site is covered with a bandage. You will be directed to keep the bandage on for several hours. You will be asked to stay at the Blood Center for 10–15 minutes after donation and offered light refreshments.

<u>Study Visits number 2, 3, 4 (one day)</u>

Platelet Reinfusion

On a date (4 to 20 days) after your blood collection, you will be asked to return to the Blood Center. On this day there will be 3 separate visits.

1. Study Visit #2 - allow 30 minutes. In the morning, the following will be done:
    - Blood pressure, heart rate and temperature will be recorded.
    - A review of health status and medication use.
    - A small blood sample (1 teaspoon) will be drawn for a baseline blood cell count.
    - If you are a pre-menopausal woman, we will collect 5 mL (1 teaspoon) for a serum pregnancy test.
    - A larger blood sample (43 mL, about 3 tablespoons) will be drawn to get a fresh sample of your platelets.

2. Study Visit #3– allow 45 minutes. You will be asked to return in about 4 hours for the following:
    - Blood pressure, heart rate, temperature, height and weight will be recorded.
    - The skin on the inside of your arm will be cleaned with an antiseptic and a needle will be inserted through the skin into a vein. A tourniquet or blood pressure cuff will be placed on your upper arm and inflated.

- A 10 mL blood sample (2 teaspoons) will be drawn.
- The tourniquet will be removed or cuff deflated
- A small amount of sterile salt water (normal saline) will be infused to make sure the needle is well placed in your vein.
- A small portion (about 3 teaspoons) of your radiolabeled stored platelets and a small portion (about 3 teaspoons) of your radiolabeled fresh platelets will be infused into your vein*.
- A small amount of normal saline will be infused to flush the line.
- The needle will be removed

* Both your stored and fresh platelets will each be mixed with a different radioactive material. The two radioisotopes used in this study are radioactive chromium (Cr-51) and radioactive indium (In-111). The radioactive tagging allows us to follow the platelets in your body's circulation. Using two different radioactive materials means we can distinguish your stored platelets from your fresh platelets.

3. Study Visit #4 – allow 15 minutes. One hour after reinfusion of your platelets, you will return to the Blood Center for the following:
    - About 2 teaspoons of blood will be collected.
    - A review of symptoms and health status.
    - Blood pressure, heart rate and temperature will be taken.

Follow-Up Blood Draws

- Study Visits #5 (Day 1), #6 (Day 2), #7 (Day 3), #8 (Day 5), and #9 (Day 6 or 7 or 8) #10 (Day 10, 11, or 12) – Allow 15 minutes. The following will be performed:
    - A blood sample of about 2 teaspoons will be collected.

The amount of radioactivity from your follow up blood samples will be measured to see how many of your refrigerated platelets survived compared to your fresh platelets.

Testing Stored and Fresh Platelets

Samples will be taken from your donated unit of blood after collection and at the end of storage. Platelets from these samples will be tested using a number of laboratory tests. These tests will provide information about how many platelets are in your blood and how well your platelets function before and after refrigeration. These tests will be done at the Puget Sound Blood Center, the University of Washington, or the US Army Institute of Surgical Research in Texas. Your blood will be kept and used for testing until this study is over. Then your blood will be discarded.

Total Blood Collected

The total amount of blood that will be collected during the course of the study is about 628 mL (slightly less than 3 cups).

Medications

You will be asked to report any changes in your medications while you are in this study. Please inform the research staff before starting any new medicines, including aspirin or aspirin-containing medications.

Blood Donations

You may not donate blood during your time in this study. You should not donate blood for at least 56 days (2 months) after receiving the radioactive reinfusion.

Birth Control

The radiation from radioactive Chromium (Cr-51) or radioactive Indium (In-111) may be harmful to a fetus. If you are a woman who could become pregnant, you must have a pregnancy test done before your reinfusion. If you are pregnant, you may not take part in this study. If you will be sexually active, you must use a method to prevent pregnancy during this study. This applies to both men and women.

RISKS, STRESS, OR DISCOMFORT

Blood Drawing

With any blood donation, the insertion of the needle to draw blood may cause temporary pain or an infection at the site of entry. During the needle stick blood may escape from your vein into the surrounding tissue. This may produce bruising, swelling, tenderness and soreness that could last for a few days. During or following a donation you may experience a reaction such as feeling faint or lightheaded, sick to your stomach, or vomiting. More serious, but rare complications like nerve injury may occur if the needle damages a nerve during the needle stick.

Risks of Bacterial Contamination from Reinfusing Stored Platelets

If the skin cleaning does not kill all the bacteria before the needle stick, or if there is a break in the collection system, bacteria can enter the storage bag. The bacteria may multiply during storage. If bacteria are present in the platelets at the time you get them, you may develop a severe infection. In the unlikely event that you get a bacterial infection as a result of the platelet reinfusion, you may need to be hospitalized for antibiotic treatment. If a severe infection develops and the bacteria are resistant to antibiotic treatment, this may cause death. This is very unlikely. Samples from your stored blood will be tested for bacterial contamination by several methods. If there is any sign of bacteria in your stored blood, it will be discarded and you will be withdrawn from the study. Signs and symptoms of an infection can include redness at the needle site, fever, chills, weakness, fainting, nausea, and vomiting. If you are experiencing any of these symptoms or think you may have an infection, call the 24 hour Emergency Number on the front of this consent form.

Risks associated with Misidentification of Platelet Products

There is a very remote possibility that you might get another subject's platelet product. This could transmit an infection, such as hepatitis or AIDS. Your collected blood product is labeled

with a unique number. You will be asked to verify your identification (name and date of birth) at the time of donation and again before you get your platelets back.

Risks of Transfusion Reactions
In this study you are receiving your own platelets. It is very unlikely that you would have a transfusion reaction. Signs and symptoms of a mild transfusion reaction include fever, chills, shaking, and rash. These symptoms would occur during and/or shortly after you get your platelets. Research personnel will be watching you during the reinfusion of your platelets and for a short time after.

Risks associated with the Use of Radiation in this Study

There are some risks from the radioactive substances mixed with your platelets. If you live in the U.S., you get about 3 millisieverts of radiation each year. It comes from space and the earth around you. This is called "background radiation. A "millisievert" (mSv) is a unit used for measuring radiation dose. The dose of radiation to the whole body from your chromium (Cr-51) transfusion will be about 0.11 mSv. The dose from your indium (In-111) transfusion will be about 0.17 mSv. The risk of harm from this amount of radiation is low. If you have more procedures that expose you to radiation, this risk will go up.

Risks associated with Radiation and Pregnancy

The radiation from radioactive Chromium (Cr-51) or radioactive Indium (In-111) may be harmful to a fetus. Both men and women able to have children should either not have sexual relations or use a method of birth control that is approved by your doctor during the course of this study. You must notify Dr. Sherrill J. Slichter at (206) 292-6540 immediately if you become pregnant, or suspect you have caused a pregnancy. The study doctor may wish to follow the outcome of the pregnancy and may need to report this to the study sponsor.

Risks associated with the use of Radiation in Future Studies

It is recommended that you limit your participation in studies like this using radioactive material. The recommended limit is no more than 4 times during a twelve-month period. These studies would have a similar use of radioisotopes (Indium-111 and Chromium-51). For each study you would be required to read and sign a new consent form prior to participating. You may decline to participate in this study as well as any future studies.

What measures are taken to minimize risks?

- The dose of radioactivity will be low – This is the same amount of radiation as that from 3 sets of chest x-rays. It is also the same amount that you would get in 10 airplane trips from New York to Seattle.
- Study staff are trained and will use procedures that minimize the chance of contaminating the platelets.
- Your platelet identification information will be verified with you so that you will get your own platelets.

- The staff collecting your blood and performing the transfusion are trained in all these procedures.
- Trained staff, as well as medical emergency equipment located in the Puget Sound Blood Center, will be available during reinfusion of your platelets to handle medical emergencies.
- If you are a pre-menopausal woman, we will perform a pregnancy test to make sure that you are not pregnant before you get your radiolabeled platelets.

Every effort is made to ensure your safety during these study procedures. However, as with any study there may be unexpected side effects as a result of your participation. If you think you are having a side effect from this study, call the Emergency Number on the front of this consent.

ALTERNATIVES TO TAKING PART IN THIS STUDY

Being in our study is voluntary. An alternative is not to participate in the research study. You do not have to be in our study if you do not want to be. You may stop at any time. Whether you choose to be in the study or not, it will not affect your future relationship with Puget Sound Blood Center.

BENEFITS OF THE STUDY

There are no direct benefits to you from being in this study. The results from this study may help patients in the future by improving the availability of platelets.

SOURCE OF FUNDING

The Puget Sound Blood Center is receiving financial support from the Department of Defense to conduct this study.

CONFIDENTIALITY OF RESEARCH INFORMATION

During the study, all records that identify you will be kept confidential. Your information will not be made publicly available except under certain circumstances required by law. If we learn that you intend to harm yourself or others, we must report that to the authorities.

Government or university staff sometimes review studies such as this one to make sure they are being done safely and legally. If a review takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm. The U.S. Food and Drug Administration reserves the right to review study data that may contain identifying information. The information collected may be published in scientific journals or summarized at scientific meetings. Your identity will be protected and indicated only by a code number.

The following groups or people will have access to your information, including your original study records and data collected during the study. By signing this form you are allowing these people to see your records and use the collected data:

- The Study Research Monitor: Puget Sound Blood Center staff that do not belong to the study will monitor study progress. They will check to see that the data being collected is correct. The monitor may observe you during your study participation.
- The University of Washington Human Subjects Division (including Puget Sound Blood Center), and the FDA: People from these groups will check that the data are correct and that the study is done properly.
- The U.S. Army Medical Research and Materiel Command (USAMRMC): This group is part of the Department of Defense and is funding this study. People from this group are allowed to review your research records in order to protect people who volunteer for research studies like this one.

Although the people listed above are allowed to see your original study records, the data collected for this study will not identify you. Your data will be given an ID code that can only be traced to your name by the research staff. All information you give will be linked to your identity by a one-of-a-kind study number. The list that links your identity to this study will be kept in a secure and locked file. Only authorized members of the study team will be able to see it. The link between your information and the collected study data will be destroyed within 10 years from when you sign this consent form.

As part of this study you will have your individual information entered in the Puget Sound Blood Center computerized Donor Registration system. This system will create a one-of-a-kind blood unit number. The number is used to track and match your unit of blood to you. Your Puget Sound Blood Center record will show that you have donated blood for 'Research Use Only'. The link between your individual identity and your 'Research Use Only' unit will be kept indefinitely in the Puget Sound Blood Center Donor Registration system.

Blood samples that are tested by off-site laboratories will be identified by study ID number only. Your name, date of birth and other personal information will not be associated with your blood samples.

We will make every effort to keep your information confidential. However, no system protecting your confidentiality can be completely secure. It is still possible that someone could find out you were in this study and could find out information about you.

OTHER INFORMATION

You may refuse to participate and you are free to withdraw from this study at any time without penalty or loss of benefits to which you are otherwise entitled.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

You will get a copy of this signed consent form.

You will be told about any new information from this or other studies that may affect your health, welfare, or willingness to stay in this study.

The study investigators may remove you from the study for any reason at any time. Their reasons may include maintaining your safety, if funding is stopped, or if new information becomes available. You may refuse to answer any question or refuse any test.

COMPENSATION FOR YOUR PARTICIPATION IN THIS STUDY

You will get $300 for being in this study. If you are not able to finish the entire study, or if you are withdrawn from the study, you will get payment for your time involved. Below is the payment scale for the procedures involved in this study:

Initial screening (Visit 1a)		$25
Blood collection (Visit 1b)		$50
Collection of 43 mL of whole blood (Visit 2)		$25
Reinfusion of fresh and stored platelets (Visit 3)		$25
First follow-up blood sample 1- 2 hours post platelet infusion (Visit 4)		$25
Follow up blood draws (Visits 5-10) $25 each		$150
Total for completing all study procedures	=	$300

You will be paid these amounts per visit to compensate you for your travel costs and time. If you finish all study procedures, you will get a check at your final blood draw visit. If you are withdrawn from the study, a check will be mailed to you.

If you get payments for being a part of this research study, you will be asked to fill out an Internal Revenue Service (IRS) W-9 Tax Form. The amount you get may count as income and may affect your income taxes. Your social security number will be required on the IRS form. There is no cost to you for participation in this research study. All research procedures are free of charge.

COMPENSATION FOR INJURY

What medical care is available in case of injury?

In the event that you become ill or injured from being in this research study, the Puget Sound Blood Center will provide emergency medical care as required. In the event of requiring more medical care, you will be referred to your doctor or transferred to a local hospital.

Funds are not available to cover the costs of any on-going medical care. You are responsible for the cost of any non-research related care. However, the Puget Sound Blood Center will pay up to $10,000 for out of pocket expenses related to the treatment of physical injury or illness resulting from the study. Tests, procedures or other costs incurred solely for the purposes of this study will also not be your financial responsibility. If you have questions about your medical bill related to study participation, contact Dr. Sherrill J. Slichter at (206) 292-6540.

Do not sign this form until you have had the chance to ask questions and all your questions have been answered.

Nothing in this consent form waives any legal rights you may have nor does it release the investigator, the sponsor, the institution, or its agents from liability for negligence.

If you think you have an injury or illness related to this study, contact study staff or Dr. Sherrill J. Slichter, right away at 206-292-6525. She will refer you for treatment.

Printed name of study staff obtaining consent	Signature	Date

Subject's statement

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, I can ask one of the researchers listed above. If I have questions about my rights as a research subject, I can call the University of Washington Human Subjects Division at (206) 543-0098. I give permission to the researchers to use my study records as described in this consent form. I will get a copy of this consent form.

Printed name of subject	Signature of subject	Date

Copies to: Subject
Subject's Study Record

APPROVED

MAY 09 2013

UW Human Subjects
Review Committee

UNIVERSITY OF WASHINGTON

Human Subjects Division
Box 359470
HUMAN SUBJECTS REVIEW COMMITTEE APPLICATION

BOX FOR COMMITTEE USE ONLY
MASTER ☐ COMM. ☐ INVESTIGATOR ☐
APPLICATION NO. 44783

☐ **Check this box if your project falls into one or more of the minimal risk ("expedited") categories of research (see web site for listing of categories) and send us only two copies of all your materials.**

I. PRINCIPAL INVESTIGATOR (Provide all the information requested. Correspondence will be directed to this person. You may designate a contact person other than yourself in section II., below.)

Name Sherrill J. Slichter Title MD Position Professor of Medicine

Home Institution (source of paycheck) Puget Sound Blood Center

RECEIVED
Human Subjects Division
MAR 14 2013

Home UW Department (if applicable) Medicine Division Hematology

UW Position or appointment (choose the most appropriate one):

Faculty: [X] Regular Faculty Appointment [ ] Research Faculty Appointment [] Clinical Faculty Appointment
[ ] Visiting Faculty Appointment [ ] Dual Appointment with PNNL
[ ] Other (describe):

UW

Student: [ ] Matriculated Undergraduate Student [ ] Graduate or Professional Student (matriculated or approved "On Leave") [ ] WWAMI Student

[ ] Resident or Fellow at the UW or Local VA [ ] UW Administration or Staff [ ] None

Mail box or address Puget Sound Blood Center, 921 Terry Ave., Seattle, WA 9810

Telephone 206-292-6540 Fax 206-292-8030 e-mail sherrills@psbc.org

II. CONTACT PERSON (Provide all the information requested.)

Name Lynda Fitzpatrick Title RN Position Clinical Research Nurse

Mail box or address Puget Sound Blood Center, 921 Terry Ave., Seattle, WA 9810

Telephone 206-292-2347 Fax 866-791-4098 e-mail lyndaf@psbc.org

III. TITLE OF PROJECT: Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

IV. SIGNATURES: The undersigned acknowledge that: 1. this application is an accurate and complete description of the proposed research; 2. the research will be conducted in compliance with the recommendations of and only after approval has been received from the Human Subjects Review Committee (HSRC). The lead research is responsible for all aspects of this research, including: reporting any serious adverse events or problems to the HSRC, requesting prior HSRC approval for modifications, and requesting continuing review and approval.

A. Investigator: Sherrill J. Slichter, MD
TYPED NAME PLUS SIGNATURE DATE

B. Faculty sponsor (for student):
TYPED NAME PLUS SIGNATURE DATE

C. The Chair, Dean, or Director acknowledges the researcher is qualified to do the research, sufficient resources will be available, and (if no external funding review occurred) there was an internal review of scientific merit.
Henry Rosen, MD

Zane A. Brown, MD
HUMAN SUBJECTS REVIEW COMMITTEE SIGNATURE

4-24-13
DATE

APPROVE ☒ DISAPPROVE ☐

Subject to the following conditions: Conditional Approval
Verified 5-9-13

Period of approval is one year, from 4-24-13 through 4-23-14

☐ Subject numbers are approved as described in this Human Subjects Review Application unless otherwise indicated above in "Subject to the following conditions" or in an accompanying letter.

VALID ONLY AS LONG AS APPROVED PROCEDURES ARE FOLLOWED

University of Washington Correspondence

INTERDEPARTMENTAL

Office of Research
Human Subjects Division Box 359470

29 April 2013

Dr. Sherrill J. Slichter
Department of Medicine
Division of Hematology (Puget Sound Blood Center)
Box 359190

Re:	Application number:	44783
	Application title:	Assessment of Whole Blood Cold Stored Platelets (Brrr Study)
	IRB Review date:	24 April 2013
	Application type:	New Application
	Approval type:	Conditional Approval

Dear Dr. Slichter,

Human Subjects IRB Committee A reviewed the above-referenced application.

Your application has received CONDITIONAL APPROVAL. This means that you may hire and train study staff, and develop or refine questionnaires, surveys, tests, and/or other similar study materials, but **you may NOT access funding or start your research at this time.** The IRB has minor conditions or requests for clarification described on the following pages of this letter which must be met before you may begin your research.

Please submit your response to this letter on the *Conditional Approval Response Form,* which is found on our forms page at http://www.washington.edu/research/hsd/docs/321
The form also includes submission instructions.

To help us better track your response, open the IRB review letter in an electronic format, and then write your answers to each IRB point directly under each corresponding question.

Your *Conditional Approval Response Form* must be received by the Human Subjects Division Office sixty days (60) from the date of this letter. **The IRB will close your new application if your response is not received within sixty (60) days.** Once we have received your *Conditional Approval Response Form* it may be reviewed by a Subcommittee or may be assigned for full Committee review,

If you have any questions please contact Lyn Brighid O'Doran, administrator for Committee A, at 206-685-9379. Thank you for your prompt response.

Sincerely,

Zane A Brown, MD
Chair, on behalf of Committee A

Lyn Brighid O'Doran
Administrator, on behalf of Committee A

ZAB/lbd

Conditions of Approval

1. Please note that you are approved to enroll sufficient subjects to have a total of 56 complete the study. HSD no longer counts the number of enrolled subjects, but rather the number of subjects that have completed the study. Please confirm your understanding of this point.

   We understand that we are approved to enroll sufficient subjects to complete study endpoints. Subjects who do not complete all observations or those whose lab results are not evaluable will be considered removed, withdrawn or lost to follow-up. Those subjects will not count towards the total of 56 subjects that have completed the study.

2. Please confirm that the Dept. of Defense (Army) is aware that Sarah Ruuska will be acting as a study monitor rather than as a medical monitor (section 5.2.5 of the DOD Supplement).

   Once our study has full IRB approval it will be submitted to the USAMRMC Office of Research Protections (ORP) Human Research Protections Office (HRPO). This submission will include information about Ms. Ruuska's role as study monitor. The reference to a 'medical' monitor has been removed from the study protocol.

3. Revisions to the consent form:
   a. Under Study Visit 1, on the bullet point about NSAIDs, please add, "such as ibuprofen or naproxen.
   b. In that same bullet point please change "nonsteroidal inflammatory drugs to nonsteroidal anti-inflammatory drugs."
   c. In the same section, regarding anticoagulants, add apixaban to the list.
   d. Under Platelet Infusion: Study Visit #3, on the 3rd bulleted point, please define normal saline as "sterile salt water."

   We have made all the above changes to the consent. Additionally, under the Compensation for Injury section, on the last page, we deleted the sentence 'However, the Puget Sound Blood Center will be financially responsible for any study-related injuries'. We replaced that sentence with one which reads, 'PSBC will pay up to $10,000 for out of pocket expenses related to the treatment of physical injury or illness resulting from the study.' This was done to bring us in line with a Blood Center internal policy revision.

   The protocol was revised to remove the term medical monitor and update the estimated time-line for the study and an instrumentation change for platelet aggregation tests.

   Tracked changes versions of the protocol and consent are attached to the Conditional Approval Response.

UNIVERSITY of WASHINGTON
HUMAN SUBJECTS DIVISION
Box 359470
Seattle, WA 98195-9470
Phone: 206-543-0098
Fax: 206-543-9218

RESPONSE FORM
Conditional Approval

This document contains no hidden branching or guidance.

For HSD Office Use Only

- [ ] Master Copy
- [ ] IRB Working Copy
- [ ] Researcher Copy
- [ ] Full IRB Review Required
- [x] Expedited Review
- [x] Approved
- [ ] Conditional Approval
- [ ] Disapproved/Denied
- [ ] Withdrawn

Date Received:

RECEIVED
Human Subjects Division
MAY 07 2013
UW

DORA MOD # /

Date of IRB action: 5-9-13 Printed name: Lyn O'Doran

IRB Chair or Designee Signature: Lyn O. Doran

Notes:

PURPOSE and INSTRUCTIONS

Purpose: *Use this form to respond to an IRB review letter when your application has received* ***Conditional Approval****.*

Instructions:

1. *Complete the first page of this form.*
2. ***Open the IRB review letter in an electronic format, and then write your answers to IRB questions directly under each question.***
3. ***Print out the IRB review letter with your answers.***
4. ***Attach those pages to this form.***
5. *Complete supplemental form(s), if applicable to your research.*
6. *Complete the Index of attachments. If you are submitting changes to the consent and/or recruitment materials at the IRB's request, please include copies in "tracked changes."*
7. *When preparing double-sided copies, please make sure that each item (e.g. application, consent form, study instruments, etc.) begins on the front of a new piece of paper.*
8. *Collate all attachments so that you have three complete "application packets."*
9. *Use clips, not staples, on at least one submission, so that the IRB staff may easily distribute your materials to additional IRB reviewers, as needed.*
10. *Submit the original and two copies.*
11. ***Do not include a revised application form or any part of an application form** unless requested.*

If the instructions above are not followed as stated, the Human Subjects Division will not review your form.

1. Research Study Information

IRB Application Number:	IRB Committee:	IRB Review Date:
44783	A	24 April 2013

IRB Application Title: Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

IRB Application Type: (•) New Application ( ) Modification ( ) Status Report ( ) Other:

Lead Researcher Name:	Contact name:	
Sherrill J. Slichter, MD	Lynda Fitzpatrick, RN	
Name of person completing this form (if not lead researcher or contact):	Email (primary contact):	Phone (primary contact):
	lyndaf@psbc.org	206-292-2347

END PART ONE

Modification Form

Version 5.8

UNIVERSITY of WASHINGTON
Human Subjects Division, Box 359470
Seattle, WA 98195-9470
Phone: 206-543-0098
Fax: 206-543-9218

For HSD Office Use Only				Date Received:
[ ] Master Copy	[X] Approved			RECEIVED Human Subjects Division JUL 24 2013 UW
[ ] IRB Working Copy	[ ] Conditional Approval			
[ ] Researcher Copy	[ ] Noted			
[ ] Full IRB Review Required	[ ] Denied			DORA MOD # 2
[X] Expedited Review	[ ] Withdrawn			

Date of IRB Action: 7-31-13	Printed Name: Barbara Konkle, MD
IRB Chair or Designee Signature: Barbara Konkle	
Notes:	

Quick submit Instructions for Modification; More Instructions are available on the HSD Forms Page

- *When preparing double-sided copies, please make sure that each item (e.g., Modification Form, consent forms, study instruments, etc.) begins on the front of a new piece of paper.*
- *NUMBER OF COPIES: Three (3) copies. (Two copies for minimal risk.)*

Research Study Information

IRB Application #	**44783**	**IRB Committee**	**A**
IRB Application Title	**Assessment of Whole Blood Cold Stored Platelets (Brrr Study)**		
Lead Researcher Name	**Sherrill J. Slichter, MD**	**Contact Name**	**Lynda Fitzpatrick, RN**
Position and/or academic appointment	Professor of Medicine	Position and/or academic appointment	Clinical Research Nurse
Department/Division	Medicine/Hematology	Department/Division	Puget Sound Blood Center
Phone #	206-292-6541	Phone #	206-292-2347
Fax #	206-292-8030	Fax #	866-791-4098
Box #		Box #	
Street address, if applicable	921 Terry Ave, Seattle, WA 98104	Street address, if applicable	921 Terry Ave, Seattle, WA 98104
Email	sherrills@psbc.org	Email	lyndaf@psbc.org

Note for users of the UW Clinical Research Center (CRC) in the UW Medical Center on 7 South: modifications that impact resource utilization on the CRC MUST also be submitted to ITHS for review and approval prior to implementation. Email to: iths-crc@uw.edu

[ ] Person completing this form is the same as the Lead Researcher	[X] Person completing this form is the same as the Contact	
Name of Person Completing this Form: (if not Lead Researcher or Contact)	Email:	Phone:

Name and Mailing Address for all paper-based correspondence
(If blank, correspondence will be directed to contact person, or lead researcher if no contact person)

Name:	Campus Box#:	Other address if not at UW:
Lynda Fitzpatrick, RN		Puget Sound Blood Center, 921 Terry Ave, Seattle, WA 98104

REASON SUBMITTED:

[ ] Reason #1: Supplemental Form: Offsite Adverse Event Log

[X] Reason #2: Researcher or Sponsor Initiated Modification: *Check all of the types of modifications you are requesting:*

[ ] A. Purpose	[ ] H. Confidentiality of Research Data, HIPAA Authorization or Waiver of HIPAA Authorization, UW Confidentiality Agreement
[X] B. Procedures	[ ] I. Researchers and research staff
[ ] C. Populations	[ ] J. Sites or locations
[ ] D. Recruitment	[ ] K. Investigator Brochure and/or Protocol Amendment
[ ] E. Consent/assent	[ ] L. Funding
[ ] F. Waiver documentation of consent	[ ] M. Other compliance approval letters/reports (Radiation Safety Approval, Data Safety Monitoring Reports)
[ ] G. Waiver of consent	

Lynda Fitzpatrick

From:	Odam, Kimberly L CIV USARMY MEDCOM USAMRMC (US) <kimberly.l.odam.civ@mail.mil>
Sent:	Thursday, August 15, 2013 1:37 PM
To:	Sherrill Slichter
Cc:	Bane, Elena G CIV USARMY MEDCOM USAMRAA (US); Brigit Ciccarello; Bennett, Jodi H CIV USARMY MEDCOM USAMRMC (US); Brosch, Laura R CIV USARMY MEDCOM USAMRMC (US); Katopol, Kristen R CTR USARMY MEDCOM (US); Lynda Fitzpatrick; Quirin, Cheryll A CTR USARMY MEDCOM USAMITC (US); TATRC mailbox; Odam, Kimberly L CIV USARMY MEDCOM USAMRMC (US); Walther, Lori J CTR USARMY MEDCOM USAMRMC (US); 'wilbur.malloy@tatrc.org'
Subject:	A-17503 HRPO Initial Approval Memorandum (Proposal Log Number 11105004, Award Log Number W81XWH-12-1-0441)

SUBJECT: Initial Approval for the Protocol, "Assessment of Whole Blood Cold Stored Platelets (Brrr Study)," Submitted by Sherrill J. Slichter, MD, Puget Sound Blood Center, Seattle, Washington in Support of the Proposal, "Pathogen-Reduced, Plasmalyte-Extended Stored Platelets," Proposal Log Number 11105004, Award Log Number W81XWH-12-1-0441, HRPO Log Number A-17503

1. The subject protocol was initially approved with conditions on 24 April 2013; fully approval on 9 May 2013; and protocol modification approved on 31 July 2013 by the University of Washington Institutional Review Board (IRB). This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements.

2. This greater than minimal risk study is approved for enrolling 80 subjects to net 56 subjects who complete the study.

3. The Principal Investigator has a duty and responsibility to foster open and honest communication with research subjects. The USAMRMC strongly encourages the Principal Investigator to provide subjects with a copy of the research protocol, if requested, with proprietary and personal information redacted as needed.

4. Please note that a Research Monitor (RM) is required to be involved in DOD-supported research studies that are determined to pose more than minimal risk to subjects (DOD Instruction 3216.02, Nov 2011). If the duties of the RM could require disclosure of subjects' Protected Health Information outside a covered entity (i.e., the RM is not an agent of the covered entity), your institution may require the identity and location of the RM to be described in the study Health Information Portability and Accountability Act authorization.

5. Please note the following reporting obligations. **Failure to comply could result in suspension of funding.**

a. Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

b. All unanticipated problems involving risk to subjects or others must be promptly reported by phone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

c. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

d. Events or protocol reports received by the HRPO that do not meet reporting requirements identified within this memorandum will be included in the HRPO study file but will not be acknowledged.

e. A copy of the continuing review report and the re-approval notification by the University of Washington IRB must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the current approval by the University of Washington IRB expires on 23 April 2014. Please note that the HRPO conducts random audits at the time of continuing review and additional information and documentation may be requested at that time. At the time of continuing review, a summary of amendments must be submitted for inclusion into the study file.

f. The final study report submitted to the University of Washington IRB, including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

g. The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this research; the issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any regulatory agencies including legal or medical actions; and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

6. **Please note**: The USAMRMC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

7. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer/Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

8. The HRPO point of contact for this study is Lori J. Walther, RN, MSN, CCRP, PMP, Human Subjects Protection Scientist, at 301-619-2286/lori.j.walther.ctr@mail.mil.

KIMBERLY L. ODAM, MS, CIP
Human Subjects Protection Scientist
Human Research Protection Office
Office of Research Protections
US Army Medical Research and Materiel Command

Note: The official copy of this memo is housed with the protocol file at the Office of Research Protections, Human Research Protection Office, 810 Schreider Street, Fort Detrick, MD 21702-5000. Signed copies will be provided upon request.

Approval to Use Radiation with Human Research Subjects
at UW or SCCA Licensed Facilities

HSRAC Form 3 (08/10)

1. *AUTHORIZED INVESTIGATOR:*	**Sherrill J. Slichter, M.D.**		*HSRAC Approval No.:* **0379-33**
Title: Professor	*Address:* Box 359190		*HSRAC Classification:* **HSRAM**
Dept. Puget Sound Blood Center	*Phone:* 2-6541		*New:* ☒ *Renew:* ☐ *Amend:* ☐

2. *TITLE OF USE:*

Assessment of Whole Blood Cold-Stored Platelets (Brrr Study)
(DoD/TATRC/MRMC Proposal 11105004) [HS# 44783 A]

In accordance with the statements and representations in your Application to Use Radiation with Human Subjects dated **2/25/13, 3/4/13, 3/14/13, 3/22/13, and 3/25/13 (2013-090)** *, approval is granted for the use of radiation in the procedures designated below in accordance with University regulations and such other conditions as are herein specified.*

3. *DESCRIPTION OF RADIATION USE:*

This is a dual-label study (using 20 μCi of Cr-51 and 10-15 μCi of In-111 in normal volunteers) of the viability of platelets derived from whole-blood units that have been stored at 4°C for between 4 and 21 days. The viability of platelets that have been derived from stored blood will be compared with the viability of platelets derived from fresh blood. Subjects will have blood samples withdrawn before transfusion of both sets of radiolabeled platelets, within two hours after transfusion, and then six times over the next 10 days. Subjects may repeat this study only 4 times during a 12-month period.

It has been established that the research use of radiation described for this project will involve only minimal radiation exposure and risk to individual subjects.

4. *CONDITIONS:*

1) Because the radiological procedures associated with this study are being conducted at a facility that cannot be administratively controlled by UW or SCCA Radiation Safety programs, this authorization is issued in an advisory sense only.
2) No subject shall receive more than 4 administrations of dual-labeled platelets during one calendar year.

APPROVAL for the use of radiation as described at UW or SCCA licensed facilities is granted by:

Rick H. Hudson, M.S., Compliance Analyst — *Date:* 27 March 2013

Kalpana Kanal, Ph.D, Associate Professor — *Date:* 28 March 2013

This approval must be renewed annually, concurrent with the renewal by the appropriate IRB (UW-IRB, CC-IRB, FHCRC-IRB, WIRB, SCH-IRB, etc. Use HSRAC Form 2.

cc: *UW-IRB* ☒ *CC-IRB* ☐ *FHCRC-IRB* ☐ *SCH-IRB* ☐ *Other* ☐

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #1		RECEIVED Human Subjects Division
Consent and Eligibility Screening		MAR 14 2013
Study ID #		UW

Consent			
Subject signed Informed Consent? ☐ Yes ☐ No	**Date consent signed**	(mm/dd/yyyy)	
Eligibility Screening			
1. Does the subject meet the Puget Sound Blood Center's criteria for a whole blood donation as defined by the Blood Center's Standard Operating Procedure and documented on PSBC's DHQ?		☐ Yes	☐ No
2. Does the subject meet study specific screening criteria as described in the protocol and documented on the Consent and Screening Worksheet?		☐ Yes	☐ No
3. Is the subject eligible for study participation?		☐ Yes	☐ No

Completed By	**Date**	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #2		
Whole Blood Collection		
Study ID #		

WHOLE BLOOD COLLECTION				
Collection Date	(mm/dd/yyyy)	**Total Whole Blood Volume Collected**	mL	
Start time	(hh:mm)	Was 500 mL ±10% collected into a Terumo CPD collection bag?	☐ Yes	☐ No
End time	(hh:mm)			
DIVERSION POUCH CBC				
Platelet Count	$10^3/mm^3$	Is the Platelet Count ≥150,000/µL?	☐ Yes	☐ No
Hematocrit	%	Is the Hematocrit ≥38%?	☐ Yes	☐ No

ASSIGNED STORAGE INTERVAL			
☐ 3 days	☐ 8 days	☐ 13 days	☐ 18 days
☐ 4 days	☐ 9 days	☐ 14 days	☐ 19 days
☐ 5 days	☐ 10 days	☐ 15 days	☐ 20 days
☐ 6 days	☐ 11 days	☐ 16 days	☐ 21 days
☐ 7 days	☐ 12 days	☐ 17 days	

Completed By	**Date**	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #3		
Whole Blood Storage		
Study ID #		

STORAGE OF WHOLE BLOOD UNIT			
	Date (mm/dd/yyyy)	**Time** (hh:mm)	**Post Aliquot Weight** (g)
50 mL aliquot removed from collection bag			
Unit placed into 4°C refrigeration			
Unit removed from 4°C refrigeration			
Unit processed to PRP for platelet radiolabeling (Centrifuge start time)			

Was temperature maintained within range for duration of unit storage? ☐ Yes ☐ No, explain below:

Completed By	**Date**	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)	
CASE REPORT FORM #4	
PSBC Transfusion Platelet Research Lab	
Study ID #	

STUDY DAY	DATE SAMPLE PROCESSED (mm/dd/yyyy)	TIME SAMPLE PROCESSED (hh:mm)	WHOLE BLOOD				
			PLATELET COUNT ($10^3/mm^3$)	VOLUME (mL)	YIELD ($x\ 10^{11}$)	WBCs ($10^3/mm^3$)	MPV fL
Whole Blood Collection Day							
End of Storage							

STUDY DAY	DATE TESTED (mm/dd/yyyy)	TIME TESTED (hh:mm)	BLOOD GAS @ 37°C on PRP			
			pO2 (mmHg)	pCO2 (mmHg)	HCO3 (mmol/L)	pH
Whole Blood Collection Day						
End of Storage						

STUDY DAY	DATE TESTED (mm/dd/yyyy)	TIME TESTED (hh:mm)	PLATELET ASSAYS						
			MORPHOLOGY (Kunicki Score 0-400)	ESC (%)	HSR (%)	MICROPARTICLES (%)	MICROPARTICLES (MP/µL)	ANNEXIN-5 (% positive)	P-SELECTIN (% positive)
Whole Blood Collection Day									
End of Storage									

STUDY DAY	DATE TESTED (mm/dd/yyyy)	TIME TESTED (hh:mm)	THROMBIN GENERATION TIME (TGT)			
			LAG TIME (Minute)	ETP (nM * Minute)	PEAK (nM Thrombin)	TIME TO PEAK (Minute)
Whole Blood Collection Day						
End of Storage						

Completed By	Date	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)
CASE REPORT FORM #5
Radiolabeling Results
Study ID Number

RADIOLABELED PLATELETS					
FRESH PLATELET		STORED PLATELET			
Fresh Platelet Label	☐ Cr-51 ☐ In-111	Stored Platelet Label	☐ Cr-51 ☐ In-111		
RECOVERY (%)	SURVIVAL (%)	RECOVERY (%)	SURVIVAL (%)	% FRESH RECOVERY	% FRESH SURVIVAL

Completed By	Date	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)	
CASE REPORT FORM #6	
Plasma Analytes Performed by Blood Research Program, US Army Institute of Surgical Research	
Study ID #	

STUDY DAY	DATE TESTED (mm/dd/yyyy)	COAGULATION			
		TAT (Range: 2.0 – 60.0 µg/L)	PAP (Range: 0 - 187 ng/mL)	sCD40L (Range: 0.8 - 5.0 ng/mL)	PAI-1 (Range: 0-100 ng/mL)
Whole Blood Collection Day					
End of Storage					

STUDY DAY	DATE TESTED (mm/dd/yyyy)	COMPLEMENT			
		C3a (Range: 33.8 - 268.1 ng/mL)	C4d (Range: 0.0 - 8.0 µg/mL)	C5a (Range: 0.0 - 1.0 ng/mL)	C5b-9 (Range: 0 - 170 ng/mL)
Whole Blood Collection Day					
End of Storage					

Completed By	Date	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #7		
Platelet Chemistries Performed by LabCorp		
Study ID #		

STUDY DAY	DATE TESTED (mm/dd/yyyy)	TIME TESTED (hh:mm)	PLATELET CHEMISTRIES	
			GLUCOSE (mg/dL)	LACTATE (mmol/L)
Whole Blood Collection Day				
End of Storage				

Completed By	**Date** (mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #8		
Platelet Thromboelastography Performed by UWMC		
Study ID #		

STUDY DAY	DATE TESTED (mm/dd/yyyy)	TIME TESTED (hh:mm)	THROMBOELASTOGRAPHY (TEG)				
			R (Minute)	K (Minute)	α (Degrees)	MA (mm)	LY30 (%)
Whole Blood Collection Day							
End of Storage							

Completed By	**Date**	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)		
CASE REPORT FORM #9		
Follow-Up Samples, Radiolabeled Platelet Reinfusion, and End of Study		
Study ID #		

Follow-Up Blood Samples: Autologous Radiolabeled Platelets		
STUDY DAY	**DATE COLLECTED** (mm/dd/yyyy)	**TIME COLLECTED** (hh:mm)
Whole Blood Collection Day		
End of Storage (PRE):Day 0		
End of Storage (POST):Day 0		
Day 1		
Day 2		
Day 3		
Day 5		
Day 7 ±1		
Day 10 ±2		

Radiolabeled Platelet Reinfusion			
Date of Infusion	(mm/dd/yyyy)	**Infusion Start Time**	(hh:mm)
Was reinfusion completed? ☐ Yes ☐ No		**Infusion End Time**	(hh:mm)

End of Study		
1. Did the subject complete the study?	☐ Yes	☐ No
2. Did the subject experience any adverse events?	☐ Yes	☐ No
3. If the subject experienced an adverse event, was the event serious?	☐ Yes	☐ No

Completed By	**Date**	(mm/dd/yyyy)

Protocol No.12-10 : Assessment of Whole Blood Cold Stored Platelets (Brrr Study)

CASE REPORT FORM #10

Adverse Events

Study ID #_____-

ADVERSE EVENTS LOG								
Adverse Event	Onset Date/ Onset Time	End Date*/ End Time	Severity Grade	Relationship to blood collection	Relationship to infusion	Action	Outcome	Serious

**Line through end date if continuing at last visit and no further follow-up was obtained.*

SEVERITY
1= Mild
2= Moderate
3= Severe
4= Potentially life-threatening

Relationship
1= unrelated
2= unlikely
3 = possibly
4 = probably
5 = definitely

Actions
1= None
2= Reinfusion discontinued
3= Referral to primary physician
4= ER/ Outpatient visit
5= Hospitalization
6= Drug administered

Outcome
1= Resolved
2= Recovered with sequelae
3= Ongoing
4= Unknown